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FRACTURE OF ARTERIES

OSKAR KLOTZ

(From the Pathological Laboratories, University of Pittsburgh, Pittsburgh, Pa.)

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FRACTURE OF ARTERIES.*

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Calcification of the media is one of the most common pathological processes found in the peripheral arteries. This condition is a degenerative process in which the tissue of the part is destroyed and replaced by an inert deposit of calcium salts. The extent of the deposit varies greatly. In its early stages we may see the antecedent degenerative changes in elastic fibers and muscle cells, followed by a gradual accumulation of calcium salts in the dead areas with the final development of a hard plaque visible to the naked eye. These patches of degeneration and deposit usually lie in the middle of the media, but again may be situated along the inner border close to the intima so that the process of calcification involves the internal elastic lamina. As the condition progresses the medial plaque continues to extend laterally in a circular fashion until an annular band encircles the artery. Such complete bands are not uncommon in the vessels of the legs and forearms.

These medial deposits are quite independent of inflammatory changes in the arterial wall, and they do not bear any direct association in their development with thickenings of the intima. In the absence of any associated pathological process in the other coats of the artery, as well as the lack of inflammation in the vicinity of the injured area, it would appear that the lesion is purely a degenerative one resulting from the stress, overstrain, and fatigue of the active components of the media. As we have said, it is the muscle tissue usually, and at times the elastic fibers, which show the earliest evidences of degeneration at a period before there is any precipitation of calcium salts. The muscle fibers often show deposits of fat droplets within them, and later a disintegration of their substance. The elastic fibrils become

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more diffuse in their staining, lose their specificity for the elastin reaction, and later accumulate granular deposits of lime.

The functional fatigue of the arterial musculature appears to result from the unusual activity of the tissues which are supplied by the particular artery. The regulation of the blood supply to any part is dependent upon the control of the blood flow by the musculature of the peripheral arteries. In a much overworked organ or tissue this control of the circulation eventually leads to fatigue of the musculature of the media, which when driven to excess, shows evidence of degeneration. The results of overtaxation of the media are more frequent in the right radial and branchial arteries than in the left. Likewise the external iliac is more markedly affected than the internal, and the femorals with their branches commonly show all grades of medial calcification in men who are active and about much upon their feet. These medial calcifications are more rarely seen in the abdominal arteries. I have repeatedly cut a sclerosed mesenteric, splenic, or renal artery expecting to find advanced medial degeneration, to be disappointed in the unusually healthy appearance of the microscopic sections, save for some fibrous tissue in the intima and adventitia, or evidence of medial hypertrophy. The coronary arteries of the heart, too, do not commonly show this lesion, and it is decidedly unusual in the vessels of the brain.

Now, although the end result in this medial lesion (calcification) is much like that in the intima of the aorta, the processes in these two tissues are quite different. In the intima of the aorta the calcareous process is preceded and also accompanied by atheroma. Thus the early precipitation of calcium salts is associated with much fat accumulation, and this usually continues, so that we find the gritty deposit mixed with a greasy and cholesterin-containing material. As the calcium salts in the intima increase, the gritty masses fuse until calcareous plaques are formed. These, however, nearly always continue to be surrounded by atheromatous material. We cannot agree with MacCordick,

who can see no difference in the nature of the calcium deposits in the media and intima. MacCordick claims that both of these calcareous deposits occur in the nature of a mortar-like material, which during life is soft and pliable. Our experience has shown that the atheromatous substance of the aortic intima is not uncommonly of a gritty, mortar-like consistence, but we have never been able to demonstrate this character in the calcareous deposits of the media of the peripheral arteries.

In the routine examination of the peripheral arteries, and particularly those of the extremities, a great many specimens are encountered showing different grades of calcareous deposits. The lesion of the media is easily recognized in the hematoxylin-stained specimens. The process being a purely degenerative one is devoid of evidence of any inflammatory exudate. Commonly the process begins in the mid-zone of the media where a small patch of muscle cells is found to have undergone necrosis. The staining quality has changed much and the involved tissue is devoid of nuclei. A curious feature in connection with the necrosis of the media with calcification is that, with the death of the muscle cells, there is a simultaneous laying down of calcium salts which obscures the necrotic tissues of the part. One finds that the area is permeated with fine dust-like granules which stain blue with hematoxylin and for the most part turn black with silver nitrate. These calcium granules encroach directly upon the neighboring muscle tissue which still appears healthy. The occasional elastic fibers, which are present in the peripheral arteries, also show the presence of calcium salts where they encroach upon the medial necrosis.

These areas of early calcification begin in focal lesions of microscopic size. Several areas of degeneration not uncommonly occupy the media within short distances of each other and, as they enlarge, fuse to form continuous streaks of necrosis, which extend laterally rather than longitudinally in the arterial coat. As the necrosis continues the progressive involvement of the media leads to the death of muscle cells in whose place the granular calcium deposit is found.

During the early stages of calcification an actual hardening of the artery is not perceptible. Where, however, necrosis is more advanced and of longer standing, and particularly where the calcareous process forms an annular ring through the media, the appearance of the involved areas differs considerably. In these advanced lesions the calcium salts no longer appear in the fine dust-like granules lying in an indefinite matrix, but they appear to fuse into a more solid mass, with crystalline characters. These crystals react less actively to the presence of hematoxylin and instead of being blue are purple, red or even colorless. The central portions of the crystals commonly give no reaction with hematoxylin.

These calcareous masses, though lying in a living tissue and representing inert foreign bodies, frequently lead to no response in the neighboring tissues. They persist for years and continue to occupy more and more of the media. As they occur in the central zone of the media, there remains a living portion of the media on both their inner and outer borders. These zones of living muscle are found even when the entire artery is encircled by a calcareous ring. In the very advanced lesions the inner zone of the media may also be occupied so that the calcified plaque is in direct contact with the internal elastic lamina. It is unusual to have the deposit of lime overstep this boundary. More commonly when calcareous masses appear within the intima they develop independently of the calcareous process in the media. It has always appeared remarkable to me that such extensive degenerative processes showed so little change in their immediate neighborhood. The muscle fibers in direct contact with the borders of these calcareous masses often show no evidence of degeneration.

Although the deposit of calcium salts may for a long time remain the only evidence of change in the arterial wall, gradually there develops in a certain number of cases a metamorphosis of the surrounding tissue in which bony plaques are found. The simple presence of small bony islands becomes increasingly more frequent with advancing age. These islands may represent an immature bony

structure of an osteoid type, but not uncommonly consist of true bone trabeculæ with calcium salts in the matrix. In the majority of instances these bony masses form irregular islands in close apposition to the calcareous deposits in the media. In many instances no evidence of trauma at the point of bone formation can be observed in the arterial wall. All authors have commented upon this association of bone development with the preceding presence of a calcareous deposit. It is, however, to be remembered that in experimental animals bone and cartilage may be induced in the arteries, in the absence of a preceding process of calcification. Harvey obtained such by painting the aorta of rabbits with silver nitrate.

In the human arteries where the bone trabeculæ develop after medial calcification there is always an antecedent process of vascularization which initiates the tissue metaplasia. Orth was among the first to call attention to this primary tissue change, which developed in the vicinity of the calcareous mass. Since then it has been described by O'Brien, Cohn, Moenckeberg, Rohmer, Bunting, and others. Some have suggested that the vascularity of the tissue represented an injury induced either through minor external factors or through the effect of irritation brought about by the presence of the calcareous deposit upon the surrounding living tissue. The reaction is commonly encountered without any evidence of an inflammatory exudate and without any sign of injury to the neighboring tissues. Fibroblasts are often seen. Numerous capillaries develop in the loose fibrous stroma immediately surrounding the calcified area. With the appearance of the blood capillaries there is a disappearance of the neighboring muscle cells, so that the calcareous structure becomes bounded by a type of granulation tissue. The capillaries are derived from the vasa vasorum. These can often be seen advancing inwards from the outer coat of the artery towards the medial degeneration. In studying this newly-developed tissue it would appear that the fibroblasts have, for the most part, their origin from the perivascular fibrous tissue of the vasa vasorum.

This process of vascularization is frequently limited to a single small area along the calcareous nodule. By no means does the entire deposit become surrounded by this granulation tissue. At times the end of an area of medial calcification alone shows this proliferative tissue change.

It is within the vascularized area lying close to the deposit of calcium salts that the osteoid tissue makes its appearance. The bony material is laid down in the interstitial tissue between the capillary loops, and the new connective tissue appears to take a prominent part in developing the matrix. Through metaplasia the connective tissue cells enter into bone formation and become the osteoblasts which are permanently arranged in the lacunæ of the new tissue. Subsequently calcium salts impregnate the groundwork. The shape of the new bone trabeculæ is in part dependent upon the presence of neighboring capillaries, around which the new structure is built.

The presence of such bone deposits without evidence of definite injury may be observed in the vessels of the extremities and occasionally in those of the thyroid, ovary, and eye. We have observed a similar reaction, though accompanied by a much greater amount of dense fibrous tissue in the vicinity of a ligature about a calcified popliteal artery. In this instance fibrosis had developed on the inner surface of the artery, as well as in the adventitia. Fragments of the former medial calcareous deposits were found in the fibrous mass and about them there were small islands of bone.

We have had an opportunity of studying a great number of specimens showing calcareous degeneration of the media. Some of these have been examined after removing the calcium salts, but the best results have been obtained when decalcification has not been carried out. It is difficult to handle this material without inducing artefacts by the ordinary methods of histological technic. Artificial fractures are easily obtained in these hard rings when cutting frozen sections. Nevertheless, as we shall point out, there is evidence that fractures of them do occur during life and may be recognized by reactionary tissue changes about the point

of fracture. The majority of the calcified peripheral vessels which we have examined were of the characters above described. The most striking feature of them was the extent of the calcareous process in the absence of reactionary changes in their vicinity. In 1914 my attention was called to a definite proliferative response in a calcified media by Prof. J. J. Mackenzie of the University of Toronto. He showed me a specimen demonstrating a fracture of a calcareous ring in the periphery artery, about which a mild inflammatory process was associated with callus formation. Since then we have studied a considerable number of arteries of old people and those from cases of diabetic gangrene and have found three in which evidences of traumatic disturbance of the calcareous process was followed by local reactionary change and callus formation.

These three specimens were obtained from elderly individuals (60, 69, 72 years). The fractures occurred in the arteries of the lower extremities, twice in the posterior tibial artery, and once in the popliteal, just behind the knee. The appearance was virtually the same in all cases. The annular calcareous deposit was of a dense crystalline character without any evidence of atheroma. At one point the ring was broken, and between the fractured ends was an organizing tissue with fibroblasts and capillaries. Close to each end of the calcareous mass was a closely-attached osteoid tissue containing osteoblasts and some calcium salts. This living bone was easily differentiated from the neighboring areas of calcification. The bony spicules were surrounded by many thin capillaries, which frequently lay in indentations in their structure. The fibroblasts appeared to have their processes enter directly into the substance of the newly-formed bone. This vascularized connective tissue formed a considerable and relatively bulky mass, both on the inner and outer surface of the fractured rings. Some of the proliferative reaction entered into the deep portion of the intima. In one specimen the area of response showed the presence of blood pigment, and in applying the iron reaction to the tissue, a positive test was obtained not only in the granular deposit of

blood pigment, but also within the bony trabeculæ. This hemorrhage had probably been a secondary occurrence subsequent to the development of the vascular callus, though it is possible that some vascular tissue had been present about the calcareous deposit prior to the fracture.

In none of the specimens was there evidence of displacement of the fractured ring. Apparently, the blood pressure within the artery restored the wall to its original shape, and the ends of the fragments were returned and in close apposition. As far as could be determined in the tissue, the soft structures lying to the inner side of the calcareous ring were uninjured, there being no thrombus deposit upon the intimal surface. It would appear that following upon the fracture a definite tissue reaction had occurred about the injury, in which fibroblasts and new-formed blood vessels were the most prominent. Associated with this response there was also a dissolution of some of the calcareous materials at the ends of the fracture, which was filled in by callus. There was nothing to indicate that these fragments had been drawn apart to leave a space at the time of injury. It appears more probable that during the formation of callus, cellular activity had also led to a removal of the calcium salts between the broken fragments. The bony structures which were found in the callus had developed by a process of metaplasia, and, similar to the bone which otherwise forms in arteries, had been closely applied to the calcium deposit.

The formation of bone within the arteries is brought about by a process not uncommonly seen in other tissues. The process is usually one of a primary calcareous degeneration, followed by an unusual vascularization around the area of deposit. It is in this vascular tissue that a metaplasia of the connective tissue leads to bone formation. This process has recently been studied in the ovary by Moschowitz.

Relatively few cases of fracture of the arteries have been described. The first of these was by Howse (1877), who reported a rupture of the axillary artery in a man of thirty-six, with extensive hemorrhage into the axilla. The patient died after twenty-five days and the torn artery was found

amidst a mass of organizing blood clot. Granulation tissue surrounded the artery, while the vessel wall itself at the point of injury grated under the knife. The calcified masses were shown to contain true bone with evidence of proliferation of the surrounding tissue. In 1886 Paul gave a very clear description of primary calcification of the media of arteries similar to the lesions described by Moenckeberg in 1903. He clearly distinguished this process from the inflammatory reactions as seen in the intima. He also noted that true ossification is not uncommon in the vessels showing simple calcareous deposits. Thromboses of the arteries, he states, frequently occur as a result of the irritation induced by the presence of the hard plates in the arterial wall. In his study he found fractures with the development of callus a common occurrence. This callus, at times, showed the presence of true bone. Bunting (1906) reporting upon the development of bone in the deep intima of the aorta of a man aged seventy-two, commented upon the evidence of trauma, hemorrhage, and inflammation in the vicinity of advanced atheromatous processes. He believed that the products of degeneration act as an irritant upon neighboring tissues and lead to the production of the granulation tissue. He also suggested that trauma may be a factor in stimulating callus about old calcareous deposits with the subsequent development of bone. In a discussion upon the development of cartilage and bone in sclerotic arteries Buerger and Oppenheimer (1908) referred to the possible influence of trauma and irritation as factors in stimulating the vascular tissue antecedent to the osseous deposit. They gave, however, no evidence of actual injury imposed upon the arterial wall. They point out that bone formation is much more frequent in the peripheral arteries than is usually recognized. Some of their illustrations suggest previous fracture in the calcareous plates. Thus we have found reference to true fracture and callus formation by only two authors.

The finding of definite fractures of calcified masses in arteries has another interesting bearing. MacCordick claimed that the calcareous deposits occurring in areas of

degeneration in arteries were not of a hard brittle nature, but in the form of a mortar-like material. This, he claimed, was the reason why surgeons were able successfully to ligate the hard arteries of elderly people. Our evidence here is to indicate the firm crystalline character of advanced medial sclerosis, not as a mortar-like paste, but in a fixed and rigid state. The presence of true fractures with callus formation is the evidence that during life the pipe-stem walls of the arteries are not apparent, but real. That the surgeon may successfully ligate these rigid arteries lies in the fact that there is healthy tissue on each side of the calcareous ring, and when his ligature closes upon and fractures the calcareous plates, the inner and outer coat of the artery are sufficient to withstand the blood pressure with little danger of spontaneous rupture. As was seen in one of our cases, organization took place in the fractures induced by ligature, not unlike the production of callus with its accompanying bone. It is, of course, to be remembered that all areas of calcification of the media do not present themselves as a crystalline deposit. In the early stages the calcium salts are laid down as a fine granular deposit, not unlike sand, through the area of degenerating muscle cells. In this stage the deposit is gritty, but I would not say mortar-like.

In two of the arteries, in which we noted the presence of fracture, the vessels were deeply seated and not readily exposed to the influence of external trauma. These vessels (posterior tibial) lie in a muscular bed where spontaneous injury may readily be received. The primary degenerative processes which eventuate in the calcareous rigidity, place the vessels at a disadvantage in their relation to the neighboring muscular structures. The sclerosed peripheral vessels lose their normal elasticity and cannot accommodate themselves to the pressure of muscular contractions of the surrounding tissues. Moreover, there is an actual lengthening of the sclerosed vessels so that their course is tortuous and no longer in the normal grooves between the muscle folds. Under these conditions the vessels may easily suffer injury

through muscular contraction and unusual bending when accommodating themselves to alteration in the pressure of the surrounding tissue. Thus it seems probable that fractures occurring in deeply-seated calcified arteries, and particularly where the vessels are surrounded by an active muscular tissue, result spontaneously as the effect of local conditions rather than from external trauma.

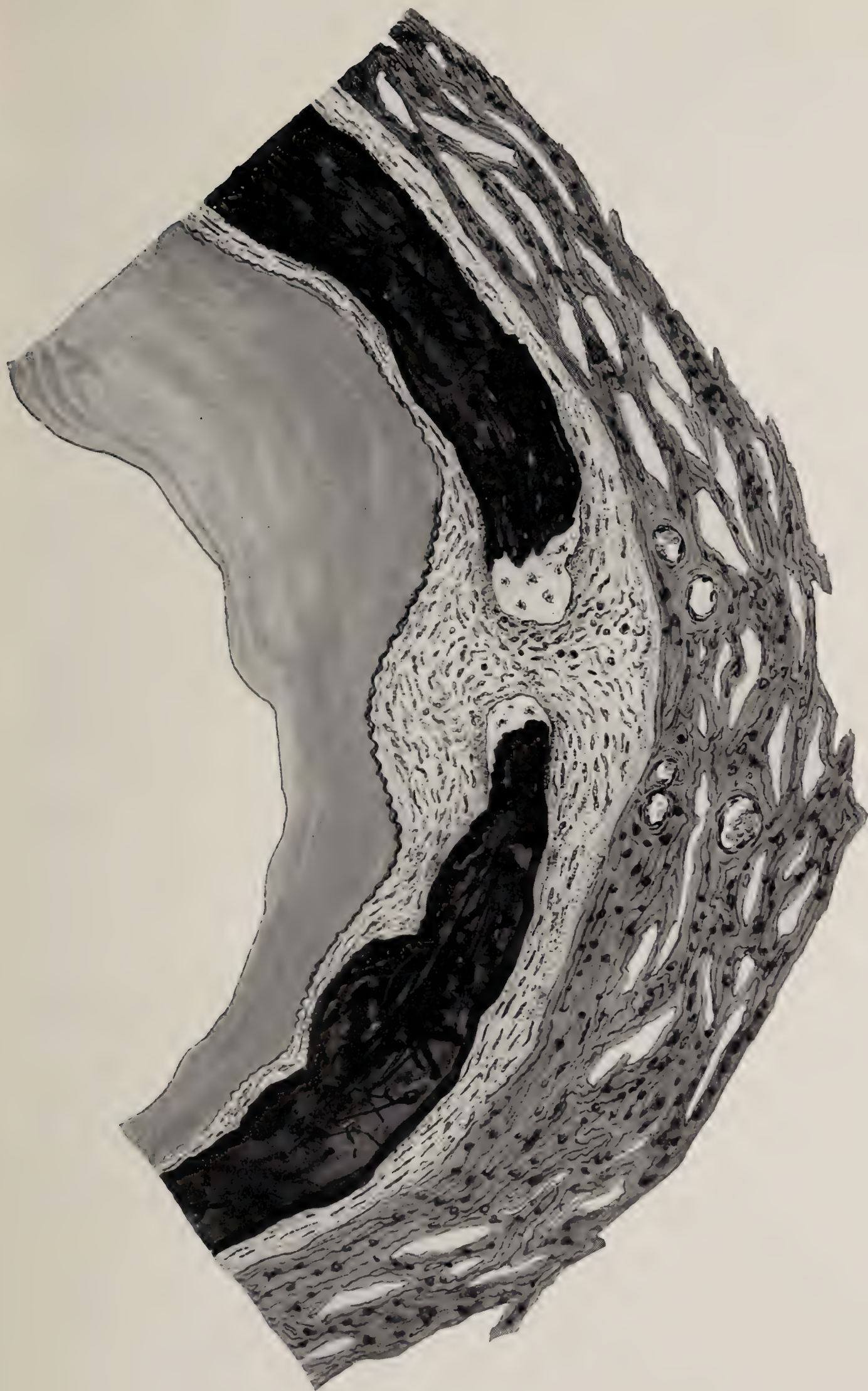
On the other hand, the fracture found in the calcified popliteal artery was probably not the result of muscular action, but due to the bending of the joint immediately in front of it. Here, however, where the vessel lies in a more exposed position, it is possible that an injury might have been received from without. Under normal conditions the arteries, as they pass various joints, lie in a loose tissue which permits them to assume easy curves during flexion, or they are placed along the outer borders of the joints where kinking of their walls cannot readily occur. As, however, the vessels become more sclerosed there is greater difficulty in moulding them for the various positions of the joint, and when an artery suffers advanced calcification acute flexion may fracture some of the calcareous rings, particularly when the tortuous course of the vessel does not permit it to move readily nor to adapt a more easy curve in the surrounding tissues. That direct trauma may lead to a fracture of a calcified artery we do not doubt, but at present it is not possible to say how frequently this actually occurs. As, however, such fractures do not necessarily entail any serious after result they give no clinical indications of their presence. In each of our cases there was no evidence that the intima had been torn and we have no history that the injury was accompanied by pain. The amount of callus which develops about the end of fractures is not sufficiently great to permit its detection in the deep-lying vessels. In the presence of a well-marked layer of living tissue on the inner side of the calcareous ring it is probably infrequent that the fracture leads to a perforation of the lumen. It is more probable that, with the distortion of the vessel at the time of injury, the inner layer of tissue becomes separated from

the calcareous masses in the media, and that with the release of the mechanical influence on the arterial wall the blood pressure smooths out the lumen.

Thus fractures of the arteries may occur through muscular activity, the flexion of joints, and direct trauma imposed upon the vessel walls. The repair of these fractures is brought about by a grade of inflammation in which fibroblasts and blood capillaries take a great part and simulate a reaction comparable to that seen in callus. These processes of repair are not uncommonly accompanied by the formation of bone at the ends of the broken calcareous rings.

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FRACTURE OF CALCAREOUS RING OF MEDIA WITH CALLUS FORMATION.

SUPERFICIAL FATTY STREAKS OF ARTERIES: AN EXPERIMENTAL STUDY

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The very many ways that have been tried to reproduce in animals lesions comparable to those found in human arteries indicate the diverse views on the causation of arteriosclerosis. In furthering such views the authors do not hesitate to proclaim the similarity of the experimental lesions with types of arteriosclerosis. Hence, one is soon struck by the great variety of lesions, both in the human and animal arteries, all of which are claimed to represent some form of arteriosclerosis. This we believe is not amiss, for it will permit the use of the term arteriosclerosis broadly, representing a group of lesions, and not an individual histological variety. This generic use of the term does not, however, appeal to all, for there are still such who would limit its use to a type unique in its mode of origin, or in its architectural character.

There remain, however, few who after investigating the problem closely can see only one causative factor. So many different agents have been demonstrated associated in the human lesions or by experimental means that we can no longer deny the harmful effects of diverse states or substances. It is futile to minimize the harmful effect upon the arteries of bacteria, intoxications, high blood pressure, changes in the character of the blood, and fatigue or "wear and tear." Obvious it is, too, that the tissue responses will not be the same. Some are proliferative, some are degenerative, while others are selective in the tissue attacked. Thus we come to recognize a great variety of lesions of the arterial

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wall, each of which represents some type or stage of the arteriosclerotic process.

We repeat, that it is only by the use of the term in the broad sense that a co-relation of the findings of the clinician and pathologist is possible. Arteriosclerosis is the group name of a variety of arterial lesions each of which produces "a hardening and thickening of the arteries," as was described by Lobstein, who coined the word.

Recently there has been considerable discussion on the effect of various substances, absorbed from the intestinal canal, upon the arteries. It has been found that the administration of certain products in the food will, when the quantities absorbed are great enough, act on a particular tissue in the arterial wall. Many articles of food have long been held responsible for tissue changes, but none have been demonstrated so clearly effective as cholesterin.

This method of investigation was inaugurated by Ignatowski, who by feeding egg yolk and brain substances to rabbits noted marked alterations in the aorta which he thought were due to severe disturbance of the animal's protein metabolism. However, the later work of Stuckey disproved this view as he obtained characteristic and marked changes in the aorta by feeding substances rich in fatty materials while he was unable to obtain positive results in the employment of egg albumin and meat. Further than this he has also demonstrated that the administration of pure neutral fats of animal and vegetable origin give uniformly negative results, while Wesselkin found no alteration in the aortas of animals fed with pure lecithin. From these findings the only substance of importance which remained uninvestigated in egg yolk was cholesterin and its esters, and subsequently it was demonstrated to be the factor which had to do with the vessel changes observed. A supposition of such nature did not appear to be very far amiss, as Chalатов had observed the laying down of much crystalline lipoid in the livers of animals fed egg yolk and brain substance. Further Aschoff noted that morphologically similar substances consisted for the greater part of combined cholesterin, while Wesselkin

had also observed combined cholesterin in the fluid crystal state in the aortas of rabbits fed with egg yolk.

From the information thus gained Anitschkow undertook a series of experiments whereby rabbits were fed daily for a period of from one to four months with from .2 to .8 gram of pure cholesterin dissolved in ten to twelve cubic centimeters of sun-flower oil, while in addition the animals received a liberal vegetable diet. In the aortas of animals so treated he found a disposition of doubly fractile fatty substances in the inner arterial layers, together with hyperplasia of these structures. From his studies he concluded that the dietetic form of arteriosclerosis was brought about by the cholesterin administration, and further that the changes induced by feeding with egg yolk and brain substances were identical with the type he described, which materials contained large amounts of cholesterin.

Our interest in this subject has been stimulated on account of the similarity of the experimental lesions with those changes which are constantly found associated with acute infections and chronic intoxications in man, namely, the occurrence of superficial fatty streaking of the larger arteries. Furthermore, the opinion of Saltykow, that only the dietetic form of the disease was worthy of the name atherosclerosis, did not seem to be well grounded and in the light of his own previous work appeared to be somewhat disconnected. Consequently a series of feeding experiments was undertaken in order to study these changes to our own satisfaction and definitely establish any similarity existing between these alterations and those found in the arteries of man.

Three series of experiments were inaugurated for this work in which rabbits were fed with cholesterin in different solvents and as a control others were fed with the solvent without the cholesterin.

The first series consisted of six rabbits which were fed with a solution of cholesterin in olive oil in the proportion of one gram of cholesterin to fifteen cubic centimeters of

the oil. This mixture was found to be a convenient one, as the cholesterin remained in solution at or about body temperature, while it crystallized out of the mixture when at a slightly lower temperature. The animals were fed daily through a stomach tube with four cubic centimeters of this mixture and in addition were given a liberal diet of hay and oats. However, this régime lasted only for a period of fifty-seven days, when after killing two animals at separate intervals and finding no gross evidence of change it was decided to double the dose for each of the remaining rabbits. The rationale of the increased dosage was supported in our next animal (Rabbit 4) which after twenty-eight days' treatment with the increased amount showed marked changes in the aorta, thus supporting our own idea, and that of Anitschkow, that the development and extent of the lesion is in direct proportion to the amount of cholesterin administered. The data for this series of animals is shown in the following table:

TABLE I.
Olive oil cholesterin.

Animal.	Days Treated.	Daily Dose of Cholesterin.	Total Cholesterin Administered.	Body Weight.		Fatty Streaks.	Microscopical.
				Beginning.	End.		
1	28	0.28 gram.	8.12 grams.	2041	2050	None.	Beginning fatty change (slight).
2	57	0.28 gram.	15.96 grams.	1870	1960	None.	No change.
3	61	0.56 gram.	17.92 grams.	2088	2690	None.	Early fatty change.
4	86	0.56 gram.	31.92 grams.	1815	1770	Well developed light yellowish streaks and raised plaques in aorta, also on aortic and mitral cusps.	Intima much thickened, made up of large cells filled mostly with anisotropic fat. Beginning proliferation of fibroblasts, splitting of elastica interna.
6	108	0.56 gram.	44.24 grams.	1418	2125	Fatty change very well marked about branches of arch. Surface rough, uneven, elevated. Pulmonary presenting the same condition near its base.	More marked accumulation of large cells than 4. Fibroblastic change well developed. Some degeneration of cells. Splitting of elastic layer and development of new fine elastic fibers in hyperplastic intima.
10	166	0.56 gram.	76.72 grams.	1364	2290	Similar to 6 but more marked, also in branches of arch and smaller pulmonary divisions aortic and mitral cusps.	The tendency to generation is not the prominent feature; rather an evenly balanced growth of large cells and supporting tissue.

The above series was controlled by feeding two rabbits daily with olive oil in the same amount as that received by the animals being fed the olive oil cholesterin mixture. These animals were given four cubic centimeters of olive oil daily for fifty-seven days and then the amount was doubled in accordance with the above experiments. Even after a period of one hundred and forty days there were no changes in the aorta or other organs of these rabbits. Thus it will be seen that the presence of cholesterin is essential to the production of these lesions, evidence which supports the work of the Russian authors (Chalatow, Wesselkin, Anitschkow).

TABLE II.

Olive oil.

Animal.	Days Treated.	Daily Dose.	Body Weight.	Macroscopic.	Microscopic.
5	87	4 cc. for 57 days. 8 cc. for 30 days.	1875	No change.	No change.
8	140	4 cc. for 57 days. 8 cc. for 83 days.	1705	No change.	No change.

With the evidence at hand that the cholesterin is the important factor in the production of the alterations found, it occurred to us that it might be administered in other combined forms. At this time one of us (Klotz) was studying the effect of cholesterin upon the hemolytic and toxic properties of sodium oleate and found that the cholesterin completely inhibited the untoward effects of the soap. It was, therefore, deemed advisable to feed the rabbits daily with a sodium oleate cholesterin emulsion containing cholesterin almost to saturation. This emulsion was made by using a five per cent solution of anhydrous sodium oleate (Merck) to which seven and one-half per cent of cholesterin were added and the mixture boiled on a water bath for several hours. This amount of cholesterin seemed to be all that could be conveniently employed as the mixture became quite gelatinous when more cholesterin was added and consequently could not be readily injected through the stomach tube.

Three rabbits were fed daily with four cubic centimeters of this mixture for a period of ten days, when the amount was doubled corresponding to the experiments in the first two series. Of the three animals in this series one (Rabbit 7) showed no alteration in its arteries at the end of eighty days. However, the second animal (Rabbit 9) killed at the end of one hundred and fifteen days presented changes in its arteries which were more advanced than any observed in the cholesterin olive oil series and even more so than Rabbit 10, which was fed one hundred and sixty-six days. The aorta of this animal (Rabbit 9) presented a most beautiful picture of fatty change in the intima. Here there was found an accumulation of material within the superficial layers causing them to bulge on the surface and giving the tissues a whitish yellow color, at times almost shiny. These areas varied in size from a pin point to raised plaques the size of a split pea. At times they were arranged in the form of fine rough streaks becoming confluent and giving the surface a general roughened appearance. This condition of fatty accumulation was most marked in the aortic arch and about the exits of the main branches, where the heaping up on the surface was so extensive that the lumen of the vessel was perceptibly narrowed. However, the condition was well marked in the remainder of the vessel and also could be followed into the iliacs, femorals, and the branches of the arch as tiny scattered whitish yellow dots. Over the surface of these areas there did not appear to be any tissue proliferation or ulcerations. In some areas the small dots looking like miliary tubercles were quite often superimposed on a general fatty surface.

In addition to the change in the aorta the pulmonary artery presented a similar interesting picture. The main trunk was greatly involved and showed many elevated whitish yellow areas in the intima. These areas varied in size and could be followed into smaller pulmonary branches as small elevated dots. A particularly large elevated area was found at the bifurcation of the right pulmonary branch.

The number of plaques in this artery was astounding and in the finer branches were diffusely distributed as raised, pin head sized dots. On section of the larger and more advanced areas in both of these vessels, thickening of the intima consisting of a light colored yellow layer was noted. In no place did we find evidence of free gruelly material or of calcification.

The fatty change in the arteries of Rabbit 10 of the cholesterol olive oil group was not as marked as in Rabbit 9. About the mouths of the main branches of the arch and also about the upper intercostals there was much change. This consisted of definitely raised, roughened, whitish yellow plaques which occupied the greater part of the surface of the vessel in the ascending portion and arch and in lesser amounts as the vessel was followed downwards. A striking feature here, as in all cases, was the extent of the change along the concavity of the arch, while the convex border was only slightly involved, a point which has been commented upon by Klotz and Manning in a study of fatty streaks in human aortas. Similar small dots could be followed upward in the branches of the arch. These areas had a peculiar shiny appearance. The pulmonary showed medium-sized whitish raised areas on the surface of the intima which could be followed into the lung but which were not as extensive as in Rabbit 9, and did not involve the small ramifications. On section the plaques on both arteries stood up prominently on the surface and could be definitely defined even in the gross. No evidence of loose or gruelly material was noted in any part nor any sign of calcification.

A detailed description of the most advanced cases in the two series of cholesterol feeding experiments is deemed advisable because it brings out several important points. The relation of the amount of cholesterol administered to the extent of the fatty change produced holds true in the individual series as the animals in the cholesterol olive oil series showed gradually progressive increase in the fatty change, each successive case having received more cholesterol than the former ones. However, another point comes

into prominence in this connection in that Rabbit 9, having been fed a total of 61.6 grams of cholesterin in the sodium oleate cholesterin emulsion over a period of one hundred and fifteen days, showed more extensive changes than Rabbit 10, which received 76.72 grams of cholesterin in olive oil during one hundred and sixty-six days. From this it would appear that the time of treatment is only one of the factors.

It does not follow from the foregoing statement that an overwhelming amount of cholesterin will produce alterations in the aorta regardless of the duration of feeding. This was proved by feeding seventy-five grams of cholesterin to a rabbit (Rabbit 12) in nineteen days without evidence in the gross or microscopical specimens of fatty accumulation in the intimal tissues of the aorta. Although this was true of the aorta, there was nevertheless evidence of a reaction on the part of the endothelial tissues in the finer capillaries of the heart and kidney, Kupffer cells of liver and the reticular structure of the spleen.

Sternberg points out that the cholesterin must be administered in a favorable form, preferably in oil, and that Aschoff and Anitschkow, who used this method, obtained results in three months, while Wacker and Hueck, employing other combinations of cholesterin, found no alterations under five months. Although we obtained positive results with cholesterin in olive oil in eighty-six days the comparison of Rabbit 9 with Rabbit 10 shows that the animal receiving the sodium oleate emulsion took less cholesterin in a shorter period and presented more marked fatty change in the intima of the vessels. Thus it would seem that the sodium oleate cholesterin emulsion was a more favorable method of administering the cholesterin. A question as to whether the sodium oleate had an irritating effect upon the internal tissues may be raised. Upon this point we have no other information than that the animals suffered no intestinal disturbance and the emulsion as used was tested for its toxic effects by intravenous injection (Klotz and Bothwell).

Again in the two most advanced cases described there was not the slightest evidence of necrosis in any of the areas of

fatty change or of the production of loose or gruelly foci in these areas, a condition which is characteristic of true atheroma and in itself signifies the gross destruction of cells. Further than this there was no extensive splitting of lipid materials as evidenced by the fact that no calcium deposits were found in the gross, a feature which is prominently connected with the development of the atheroma and calcification in man. That the splitting of lipoids with the attraction of calcium salts by the fatty acid radicals liberated is only a step in the process of calcification was demonstrated by one of us (Klotz) and more recently was definitely proved to be so in an experimental study of the effect of cholesterin oil solutions on the arteries of rabbit's lungs when the material is injected into the ear vein. It must be understood that here our discussion deals only with the macroscopic appearance, as the microscope changes have been reserved for subsequent discussion.

The last rabbit (Rabbit 11) in the sodium oleate cholesterin group received the same course of treatment as Rabbit 9 for one hundred and fifteen days and then allowed an interval of seventeen days without treatment. The intention was to allow this animal to continue living without feeding in order to note the effect of the withholding of cholesterin upon the fatty plaques in the arteries. After a period of seventeen days without cholesterin feeding the animal suddenly became paralyzed in all parts, without any sign of emaciation or other untoward symptoms and it was decided to kill it for this reason. One would expect the intimal changes in this animal should closely resemble those of Rabbit 9, as they both received 61.6 grams of cholesterin in one hundred and fifteen days. The most striking feature in contradistinction to the previous animal (Rabbit 9) was the marked diminution in the size of the fatty plaques in the aorta. Here there was evidence that a more extensive fatty accumulation had existed than was present at the time. A broad rough plaque in the arch about the origin of the vessels did not show the heaping up as seen in the plaques of the previous case. Throughout the vessel there were many

narrow and flattened streaks, indicating a previously existing severe fatty accumulation. These tiny fine markings could be followed into the branches of the arch and were now particularly noticeable at the origin of their branches. This is suggestive that the storing up of fatty materials had been more intense, and as a consequence would take some time to exhibit appreciable reparative change.

The character of the blood in these experiments is definitely altered and consists of a hypercholesterinemia as proved by Rothschild, Wacker and Hueck, Weltmann and Biach and others. The cholesterin content of the blood of Rabbit 9 was five milligrams per cubic centimeter at the time of death. Since it has been definitely demonstrated that the arterial changes are dependent upon the cholesterin in the blood, it would follow that the amount of this material in the blood stream must be continually maintained. Or better it may be looked upon as a reaction of a particular kind of tissue to an excessive amount of a substance normally present in the blood. With the cessation of this abnormal amount these particular tissues are no longer needed for the unusual metabolic activities. This is in support of what we have observed in Rabbit 11, allowed to live a period of days without cholesterin, after a treatment equal in time and amount to Rabbit 9, in which a reaction more pronounced than in any of our experiments was obtained.

Whether or not the fatty changes in the arteries of Rabbit 11 would have ultimately disappeared we are unable to say, nevertheless we have evidence that it was diminishing in extent. A further discussion of the storing up of lipoid materials in these areas will be undertaken in the consideration of the microscopic changes.

TABLE III.

Sodium oleate cholesterin.

Animal.	Days Treated.	Daily Dose.	Total Cholesterin.	Body Weight.	Fatty Streaks.	Microscopical.
7	80	0.56 gram.	42 grams.	2020	None.	None.
9	115	0.56 gram.	61.6 grams.	2155	Large, heaped up, elevated rough streaks and plaques. Present in aorta and its branches, pulmonary and its branches, aortic and mitral cusps.	Intima markedly thickened; similar to that in Series 1, but more prominent.
11	115	0.56 gram.	61.6 grams.	2270	Smaller than in 9, only on aorta and branches of arch.	Less than Rabbit 9, however, same type of cell change.
12	19	3.94 + grams.	75 grams.	2105-2080		

Before the development of the macroscopic fatty plaques there is microscopical evidence of change consisting of a thickening of the intima. In such areas cells of varying shape from round to spindle were found to form multiple layers on the surface. Within these cells there was a sudan staining material of fine granules and globules. There was no free fatty material in the intimal tissue, all fat staining particles being intracellular. In places a free granular fat occurred in the elastic layer of the intima and there was some fatty infiltration about the nuclei of occasional muscle cells of the media. The lipoid observed at this stage was small in amount and isotropic.

With the development of the fatty streak a different picture was observed. The vessel presented a much thickened intima which bulged on the surface and had a corrugated warty character due to localized proliferation of cells. The striking feature of this hyperplastic layer was that it was made up of large watery looking cells with round nuclei and clear protoplasm presenting a very delicate reticulum. However, in addition to this there was a definite increase in the fixed cells of the intima consisting in an overgrowth of elongated spindle cells with oval nuclei. Scattered through the

tissue near the surface small mononuclear cells without visible cytoplasm were occasionally seen. Near the surface the cells were quite compact and smaller than in the areas. They had round centrally placed nuclei and uniform deeply staining cytoplasm. The large watery cells were filled with finely globular lipoid material staining with sudan and presenting the doubly refractile character with Nicol's prism. Many fine spicules and needles of cholesterin were seen as well as clumps and rosettes. This latter material stained a deep blue with Nile blue sulphate, while the anisotropic globules were stained in shades from pink to blue.

Examination of sections treated with Weigert's elastic tissue reagent and hematoxylin showed the proliferative reaction to be limited to the intimal tissue inside of the internal elastic lamina. The inner elastic layer showed splitting and granulation with the development of new fibers running inwards to the intima. Fine dots of elastin arranged in short lines were seen in the intima without demonstrable connection with the main layer.

With the further development of the fatty plaque the intimal thickening became more pronounced and warty. In places the overgrowth consisted of many layers of cells with "foam like" characters. The protoplasm of these cells was made up of a delicate reticulum presenting a spongy, almost honey-comb meshwork. Some parts showed the heaping up of cells, one upon the other, resembling the structure of stratified epithelium, while others had the radiating column-like arrangement of the adrenal cortex. Distributed through this main structure occasional islands of these same cells were seen forming large groups of closely packed cells with definite limiting membranes. However, it may be stated that the column-like arrangement of the cells predominated. In general the axes of all these cells were directed at right angles to the surface of the vessel. The "foam cells" varied in size from that of an ordinary lymphocyte with a small rim of cytoplasm to very large watery cells, some of which fused to form masses containing several nuclei and in which the individual cell walls could not be made out. As the size of

these cells varied so did the shape, evidently depending upon their intimate association and amount of compression. All types were observed from round to elongated forms. Some had flattened sides and were roughly pentagonal. The nuclei of the "foam cells" were for the most part small and deeply stained. Again in some of the cells the nuclei had a pyknotic character with rough granular edges. Some of the large cells lay close to the surface of the vessel, while again elsewhere flattened cells separated them from the lumen. Over the most prominent areas there was a formation of several layers of dense tissue made up of fibroblasts with occasional lymphocytes.

Associated with the appearance of these groups of "foam cells" was also the development of fibroblasts. These formed a reticulum amidst which the foam cells were disposed. The growth of true fibrils was readily demonstrated by Mallory's phosphotungstic stain. The growth of fibroblasts was more pronounced in the deeper layers, and in the region of the internal elastic membrane the tissue was more compact. Here the cells had various shapes, some being elongated, some stellate with several processes, while others a little more round than spindle had a watery looking protoplasm like the larger foam cells. The nuclei of these cells varied from round to oval. The chromatin material was aggregated into clumps with a clear background of bluish material.

Sections treated with Weigert-van Gieson showed marked splitting of the internal elastic membrane with the production of several layers and the transverse rupture of the fibers in many places. There was marked granulation of the fibers. In one place there was an area which extended almost to the middle zone of the media where muscle and elastic fibers were entirely replaced by the downgrowth of the foam cells and their supporting fibroblastic structure. Here the elastic fibers were broken and there was a considerable gap between the split ends. At the inner elastic layer, the break was complete and was occupied by the ingrowing intimal cells, while in the media where less disturbance was evident the

fibers were broken but the granular frayed remnants lay among the cells. The broken ends bulged and were quite blurred. However, careful examination revealed fine fibrils extending outward giving a brush end. This rupture of the elastic fibers was also seen in several other places but did not involve more than two elastic layers of the media, while the above invasion had destroyed the continuity of seven layers, there being nineteen layers of elastic tissue in the vessel. The picture here reminded one not a little of the gross destruction of elastic fibers and muscle tissue in syphilitic aortitis.

On the surface of this area there was a connective tissue thickening and new elastic threads. There was evidence of beginning degeneration in the large cells underlying this area. Throughout the intimal tissue there was seen the development of many fine granular lines of elastic tissue forming a delicate network about the cells. It would appear as though these newly formed fibrils were closely associated with the fibroblasts. They were far removed from the internal elastic layer and extended close to the surface of the vessel, reminding one of Jores' description of the new development of elastic tissue in his hyperplastic form of intimal disease.

Sections stained with sudan and hematoxylin showed the large cells of the intimal tissue loaded with a lipoid material, mostly in the form of tiny globules. However, in some cells larger globules were seen which did not tend to fuse. The nucleus was rarely disturbed from its central position. Between these cells of the intima many of the supporting spindle cells contained fine globular fat in their protoplasm. At one part on the surface there was quite a thickening composed of several layers of spindle cells containing fine globular fat. This thickening on the surface was well demonstrated in the paraffin sections. The large fat cells lay together in compact clumps forming pseudo-xanthomatous masses. Some of the cells were so packed with lipoid material that the nucleus was crowded to the periphery, forming a crescentic body against the cell wall. Most of

the lipoid material was intracellular. However, in the deeper layers, in the region of the elastica interna there was a different appearance. Close to this layer the tissue was quite compact with the growth of fibroblasts and beyond this there was an hyperplasia of the musculo-elastic layer. These deeper cells and the inner cells of the muscular layer contained fatty globules. The intracellular tissue of the whole intimal coat was very loose and edematous and was sparsely sprinkled with orange staining granules. This fat is probably attracted by the peculiar character of the intercellular substance (Klotz) even when the cells of the inner muscular layer show no degeneration.

Sections stained with sudan and fuchselin demonstrated fatty degeneration of the elastic fibers. At times the foam cells were seen lying between the layers of muscle cells in the media similar to the cells of the intima with globular sudan staining material. A large number of cholesterin clefts were seen in the deep intima independent of the fat-containing cells and lying in a tissue where fibroblasts were prominent. Clefts were often seen in the foam cells of the intima. The major portion of the lipoid was anisotropic. The doubly refractive bodies were present within the cells in abundant numbers, as were also needles and crystals of cholesterin. Most of the fat stained blue with Nile blue, a little of it being tinged red. With the production of these lesions there was associated a particular type of cell and one which is evidently closely connected with the lipoid metabolism of the body. It comes into prominence and holds the attention not only because of its abundance in the arterial changes but also because of its presence in the other organs of these animals. The foam-like character which it exhibits wherever seen places it within a class of endothelial cells. Aschoff and Landau have described a system including the reticulo-endothelium of the spleen, bone-marrow, and lymph nodes and the Kupffer cells of the liver, under "endothelialer stoffwechsel apparat." They consider it a very important intermediary system in lipoid metabolism. Anitschkow believed that these cells originate from the small lymphocytes of the

blood and he designates them as cholesterin-ester phagocytes. Saltykow considered them as connective tissue converted into macrophages. Although in places it is rather difficult to distinguish between these lutein cells (Klotz) and ones looking like swollen fibroblasts, we have nevertheless been unable at any time to establish a relationship between the foam cells and connective tissue cells. With phosphotungstic acid hematoxylin it was impossible to demonstrate fibrillar production by these cells, while the fibroblasts formed definite fibroglia fibrils. However, it would appear that foam cells arise from a fixed type of cell which lies dormant in all tissues and which when called upon responds to the demands of the body overburdened by the presence of cholesterin-lipoid substances.

With the production of a hypercholesterinemia by feeding, the animal presents a definite reaction on the part of its tissues, which can be recognized not only in the gross but also by peculiar microscopical features. That the tissue proliferation is an endeavor on the part of the organism to combat a substance appearing in excess in the body fluids seems quite clear. There is evidence that the body is trying to accommodate itself to the increased amount of cholesterin in the blood. With the development of a certain degree of hypercholesterinemia this substance is excreted in the bile as indicated by Rothschild. Further, there is an attempt upon the part of the parenchymatous cells of the liver and adrenal particularly to store up this material. Chalatow has demonstrated in feeding experiments that the liver cells become filled with doubly refractive lipoid substances while Sternberg proved this point in connection with the adrenal in pregnancy and feeding experiments. Along this line there is still another tissue of endothelial origin which comes into play. The Kupffer cells of the liver show an active response to hypercholesterinemia. Rothschild and Landau have called attention to the proliferation of the Kupffer cells of the liver under conditions of increased cholesterin metabolism, while Sternberg has observed a similar increase in the endothelial cells of the adrenal cortex. These cells contain

doubly refractile lipoids in large amounts. We, ourselves, have noted the hyperplasia of the Kupffer cells in liver, of the endothelial cells in the adrenal and of a most interesting accumulation of endothelial cells in the interstitial tissue of the medulla of kidney. This latter condition will be reported upon in a separate paper. In the spleen there is a proliferation of the reticulo-endothelial structure with large phagocytic cells massed in the sinuses. The response of endothelial cells has been noted (Klotz) in connection with the injection of cholesterin into the ear vein, where the endothelial cells of the small arterioles of the lung showed marked proliferation in their attempt to deal with the cholesterin lodged in them. We have also observed a proliferation of the endothelial cells lying in the interalveolar spaces connected with the alveolar capillaries in which fine globular lipid was often seen. Thus it would seem that there is a general reaction on the part of the organism by a particular kind of tissue especially adapted for the function it has to perform. So it appears that the intima of the arteries being continually exposed to the irritating substance present in the blood shows a corresponding reaction. This change in the intima progresses as the cholesterin increases in amount and is maintained only by the continued excess of the cholesterin in the blood.

The character of these experimental lesions reminds us not a little of the superficial fatty streaks which are so commonly seen in human autopsies. On previous occasions we have commented upon such findings and discussed their significance. These superficial fatty streaks are most frequently found in the human aorta and its main branches. In the smaller arteries their position is in relation to the bifurcation of the vessels. They are, however, met with in the peripheral vessels in situations other than these and without definite anatomical relations.

In the human these superficial fatty deposits are not necessarily associated with other pathological changes of the arterial wall. They may occur in an otherwise healthy artery, but may also be seen in vessels with considerable

damage. The frequency of development, however, appears to lie with the healthy aorta of young individuals. The best examples of it are seen in the acute infections, such as typhoid fever, acute septicemia, acute intoxications following burns, pneumonia, scarlet fever, as well as in severe blood diseases and uremia. Our attention has been called to the frequency of these streaks in the aorta of typhoid patients, and it was always some surprise if marked superficial deposits were not found.

The lesion here under discussion is not to be confused with the deep atheroma. In fact, since we have shown in the experimental work that the presence of the fat in the streaks is dependent upon its storage by definite types of cells we are not dealing with true atheroma. In true atheroma much of the fat and lipoid lies extracellularly amidst a débris of tissue destruction. Nevertheless, we cannot deny that some at least of the areas of superficial fatty streaking may advance to atheroma. This occurs when by the extent of the fatty deposit death of some of the fat-containing cells takes place with the liberation of their contents into the surrounding tissue. This degenerative process in the specific type of cell is accompanied by more or less degeneration in the surrounding tissues so that a fatty change in the connective, elastic, and muscle tissues is not uncommonly seen.

This superficial lesion of the intima is the one that has been most frequently encountered by those searching for arteriosclerosis in the young. It is this lesion which we included under our discussion on the finding of arterial changes in children with scarlet fever, and it is also to be met with in diphtheria. It is probable that it is one of the tissue reactions which has been observed by those discussing presenile sclerosis. That its early stage is not a sclerosis is obvious, but, as has been previously described, the lesion may be followed by connective tissue proliferation, in which event an intimal sclerosis of the nature of an endarteritic cap is produced over the fatty area. We feel convinced that not a few of the early lesions of degeneration described by Jores as a type of destruction of the musculo-elastic layer belong to this group.

The individual lesions of the human arteries show a unique structure very similar to those in the experimental animals. The small nodular areas are composed of dense groups of large endothelial cells closely packed together and filled with fat-staining material. Neutral fat and evidence of fatty acids are easily demonstrated. Free cholesterin as well as combined cholesterin with anisotropic qualities is commonly present within these cells. The accumulation of these cellular elements is accompanied by more or less edema of the area and some lymphocytic infiltration. The latter varies greatly and we have not been able to conclude to what extent the human lesions have this inflammatory reaction. We have, however, always noted it where the intimal response accompanied an infectious disease. In the late stages more or less connective tissue hyperplasia replaces the fat containing cells. Complete disappearance of the fatty streaks is common, but their apparent loss does not indicate a restoration of the intima to its normal, for the development of connective tissue leads to a thickening which, although not observable to the naked eye, introduces a new condition to the arterial wall.

The character of these fat-containing cells we have discussed at some length in previous articles, and this we need not repeat. We would, however, point out that the results obtained in the experimental feeding though simulating closely the human lesions can easily be produced to a degree never encountered spontaneously in man. In the human arteries the fat-containing endothelial cells are arranged in clusters, streaks or plaques in the loose laminated stroma of the superficial intima. There is commonly more or less connective tissue between these plaques and the vessel lumen. On the other hand, in the animal experiments the lipoid containing endothelium develops in large nodular masses in which the cells are often arranged in parallel rows. These remind one of the rows of cells in the cortex of the adrenal. At other times their disposition is quite like that in the human artery. An inflammatory reaction is usually

wanting in the experimental lesions and the cellular hyperplasia develops to a degree occupying the whole intima or even extending into the media. This degree of proliferation we have never seen in human cases.

The similarity of the fatty streaks seen in the human aorta and those developing in rabbits with hypercholesterinemia is very striking. Their distribution and their position in the intima and the character of the cells which are the carriers of the lipoid substances are virtually identical. A difference, however, is noted in the color of the areas. Those in human arteries are of a pale yellow, while those in experimental rabbits are glistening white. This is dependent upon the amount of cholesterin present in the respective deposits. In the rabbit experiments a very much greater quantity of cholesterin is absorbed and appears in the blood than occurs in the human. The proportion of cholesterin in the feeding experiments was usually about seven per cent of the vehicle. The induced hypercholesterinemia was found to range up to five milligrams per cubic centimeter of serum. In the subsequent fatty streaks the deposit both within and without the involved cells of the intima contained much demonstrable cholesterin. Such grades of hypercholesterinemia are not commonly encountered in the human, and furthermore the quantity of cholesterin within the lipoid deposits of the arteries is not as great as found in these feeding experiments. It would appear, therefore, that the color of the fatty streaks is in part at least dependent upon the amount of cholesterin in the deposit.

It has been evident to all observing fatty streaks in human arteries that their presence is for the most part transient. Commonly as these streaks are to be found in the arteries of typhoid patients, they are not prominently present in individuals who have fully recovered from their typhoid. That these streaks do not entirely disappear has been evident to us, for we have found their remains as well as the development of a superficial connective tissue covering in not a few vessels of patients dying some years after the acute attack.

It is interesting that a similar diminution in the lipoid content of the experimental fatty streaks was observed in an animal which after having a marked hypercholesterinemia, induced for a period of one hundred and fifteen days, had been allowed an interval of several weeks during which no cholesterin had been given. This is very suggestive that abnormal quantities of fat in these particular lesions is directly dependent upon a hypercholesterinemia.

Although the fatty streaks are prone to disappear from the intima, permitting a complete restitution of its tissues, some lesions leave evidence of their presence. This as we have above stated may appear in a secondary connective tissue proliferation over the plaque. Others show the presence of the foam cells long after their development. Such cells lie embedded at various depths in the intima. Finally, however, evidence of these fatty streaks is encountered in true atheromatous deposits in the interstices of the tissues. Such deposits result from the disintegration of the fatty cells by which their contents are liberated freely into the tissue and it is not uncommon to see these deposits with a number of the foam cells remaining in their periphery. The liberated lipoid material which was originally intracellular and the cholesterin may be demonstrated both as esters as well as in free crystals. Such areas are prone to accumulate calcium salts. Although since 1905 we have repeatedly laid emphasis upon this mode of calcium accumulation, several German authors appear to have rediscovered this process since 1908.

It has been shown that the cholesterin content of the human serum averages less than 1.8 milligrams per cubic centimeter. The quantity fluctuates to some extent under normal conditions. It has been demonstrated, however, that there is definite increase in the cholesterin content of the blood under certain conditions, such as pregnancy, syphilis, cholelithiasis, diabetic lipemia, nephritis, arteriosclerosis, and convalescence of acute infections. Undoubtedly as the blood is studied a still greater variety of diseases will be included in the group. It is probable that this increased cholesterin content forms a true hypercholesterinemia for man. Whether,

however, the mere presence of an increased amount of cholesterol in the blood is the factor which leads to its deposition in the intima of arteries has not been determined. It is possible that certain irritants, frequently bacterial, are stimulators of the peculiar tissues which have to do with the vascular storage of cholesterol.

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IRON IMPREGNATION AND INCRUSTATION OF
VARIOUS TISSUES.

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The presence of iron in calcium deposits has recently had a [363] new importance attached to it in the demonstration of curious bodies believed by some to be fungi. In the intensive researches that have been made upon the various tissues in pseudo-leukæmia, attempts have been repeatedly made to demonstrate micro-organisms as causative agents. Cultural and histological methods have been applied, and in some instances success has been claimed in demonstrating bacterial types. In part, at least, it would appear that the histological methods have been most uncertain, as the development of artefacts, as well as inorganic precipitates, have suggested conclusions which closer scrutiny cannot substantiate.

Recently Sprunt has called special attention to these fallacies. In 1911 he reported peculiar findings in a case of splenomegaly associated with liver cirrhosis. The diffusely fibrosed spleen contained "ocher-colored patches" which, on microscopical examination, showed a peculiar pigment about the trabeculæ and particularly about the blood-vessels. The vessels lay amidst masses of fibrosis, and in the vessel wall, as well as to a lesser extent in the surrounding tissues, were bands and threads of golden pigment. Sprunt was able to demonstrate calcium and iron in these deposits and found that they occurred mainly in elastic tissue. The elastic fibers had undergone various grades of degeneration which then appeared to have a chemical affinity for both calcium and iron. These impregnated salts were readily removed from the tissues with dilute mineral acids.

Subsequently Gibson reported the presence of a streptothrix in the spleens of six cases of "splenic anæmia." Four of these

[363] cases were of the variety of Banti's disease. In these, as well as in the remaining two cases, he was able to demonstrate threads or strands of yellow hue, which he believed he was able to differentiate from tissue structures and recognize as streptothrix infection. Cultures which were made from one case were negative.

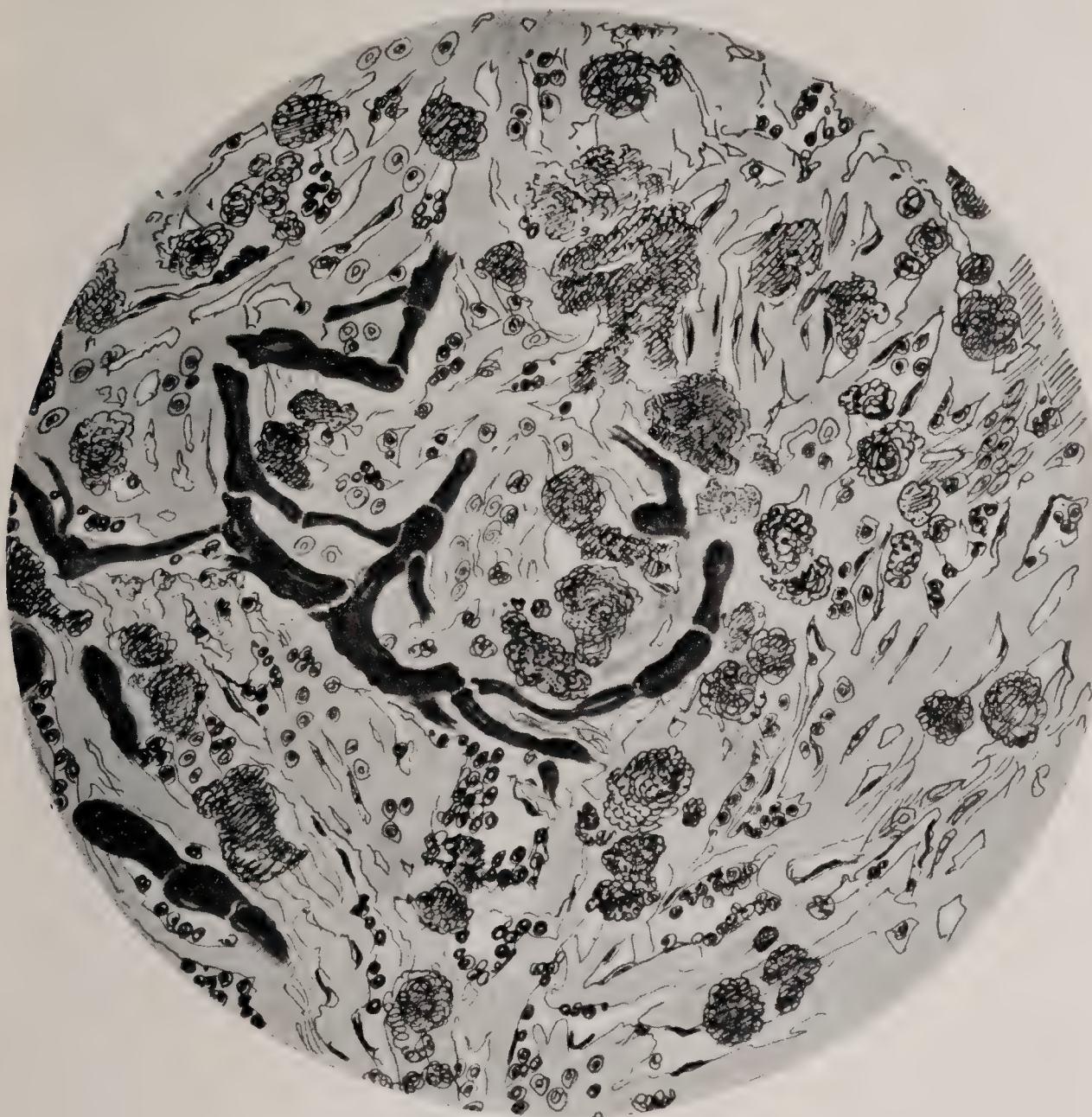
In 1915 Sprunt reported another case of splenic anæmia in a man with a history of lues and malaria. The spleen had been removed at operation. Similar pigmented patches were found among the enlarged trabeculæ, and microscopic deposits of iron and calcium were demonstrated similar to those in his previous case. Somewhat similar observations had been made in 1902 by Hektoen. He observed a process of degeneration in the elastic fibers, with subsequent incrustation with iron salts, in areas of subacute inflammation. These altered fibers were at times incorporated within giant cells. In one of the drawings of these deposits, he shows a structure very similar to the "streptothrix" described by Gibson.

Our attention was called to a similar finding in which, with the application of the simple stains alone, it appeared that we were dealing with a streptothrix or with the mycelial threads of the hyphomycetes.

The specimens were obtained from a man 46 years old, who for several years had suffered from diabetes. At autopsy, the case illustrated one of bronzed diabetes, in which the hæmato-
[364] chromatosis had involved many organs. The most marked deposit of the rusty pigment was found in the spleen, mesenteric lymph nodes, liver, pancreas and heart. As the case will be more fully discussed in a study upon bronzed diabetes, I shall not go into the details of the history or the pathological findings, save those of the spleen and lymphatic glands.

The spleen was of normal size and its capsule was thin and somewhat wrinkled. The tissues were flabby and on section they appeared quite dark with occasional rusty areas. The Malpighian bodies were visible. The pulp substance was not easily broken, but there was no definite evidence of fibrosis. The rusty areas were few in number and consisted of small irregular patches. No direct association of these areas with vascular channels could be made out.

The mesenteric lymph nodes and particularly those about [364] the head of the pancreas were a little enlarged, soft and tough. Each node was of a rusty brown color. The tissues about these lymph glands showed no evidence of pigmentation.



CALCAREO-FERRUGINOUS DEPOSITS IN LYMPH GLAND.

The lymph nodes on section showed a diffuse fibrosis throughout their structure and a peripheral thickening of the capsule. Unusually heavy trabeculae entered the gland from the capsule inwards; thus the more marked fibrosis was in the periphery of the gland. The follicular arrangement of the node had been lost and the lymphocytic elements were scattered diffusely without arrangement between the trabeculae and the reticulum. Where the fibrous tissue was laid down in heavier masses it had a very hyaline appearance and showed very few connective-

[364] tissue cells. Scattered throughout the gland structure was a heavy deposit of golden-brown pigment. This pigment was granular, varying from fine irregular dust-like particles to larger aggregated masses formed by the welding together of many small granules. The greater amount of the pigment lay free in the interstices of tissues, but some of it had been phagocyted by endothelial cells.

Aside from the remarkable deposit of granular brown pigment which was found to be iron-containing, there was also a unique appearance in the hyaline trabeculæ. In places a deposit of the iron-containing pigment was found within the clefts of this tissue. The deposits were molded into intricate forms identical in appearance with a coarse mycelium or the strands of a streptothrix. These structures showed beautiful transverse segments and not uncommonly club formation at the ends of the strands. In several places, however, one could still make out the individual granules entering into the formation of the more solid segmented strands. Branchings were frequently present, so that divisions, somewhat comparable to those of the penicillium, were found.

Although it appeared evident that all of these fungus-like structures had a common mode of origin, it was evident that some had undergone changes in their composition. Where these molded strands were in process of formation, their character was similar to that of the individual iron-containing granules which were diffusely scattered in the gland tissue. The Nishimura tests for iron were positive, both for the individual granules and also for the streptothrix-like structures. There was, however, some difference in the intensity of the reaction, in that the molded strands were of a lighter color, even blending into a greenish yellow. Furthermore, it was found that with the ordinary hæmatoxylin stain, irregular masses (by no means all) of the streptothrix-like strands gave a dark purple reaction, which shaded off into a lighter wine color. Some of the strands did not take the hæmatoxylin stain. Likewise, too, the application of aniline stains led to a partial coloration. The structures, however, did not stain by Gram's method. With silver nitrate a great number of these structures became black and were sharply outlined from the surrounding

brown granules. When sulphuric acid was used, a few acicular [364] crystals of calcium sulphate were formed. From the use of dilute mineral acids no gas bubbles were obtained. After the removal of the calcium and iron there remained a transparent hyaline matrix.

Besides these curious structures, there were other linear masses which could be distinguished from those above described. Whereas the former were found to occur in the interstices of the hyaline stroma, the latter had their origin within the more solid structures, at times in the vicinity of blood-vessels. In these there was a primary change of the tissue-matrix with a varying deposit of calcium salts. In these, fine elastic fibrils that coursed through the tissue stroma were demonstrated by Weigert's method, and evidences of varying degrees of degeneration with more diffuse staining of the fibers were obtained. They entered into the tangled structures in which calcium salts were readily demonstrable. In these calcified strands one could not see any pigment granules, and with ordinary or elastic-tissue stains one could not recognize any coloring suggesting an iron-content. Here again, however, the Nishimura test showed the presence of a diffuse iron reaction, [365] as if the iron salts had been diffusely absorbed in these areas of calcification.

Although it was noted that elastic fibrils in stages of degeneration entered into the bands of ferruginous calcification, these could not be demonstrated in all instances. At times the thickened trabeculae of the lymph gland showed a homogeneous hyaline character which, with the elastic stains, gave a diffuse and relatively pale reaction. Frequently no true fibrils were present, but only a washed or blotchy elastin reaction was obtained. In these areas iron was commonly demonstrated and occasionally small granules of calcium salts.

Similar structures were observed in the spleen. These in part were aggregated in the trabeculae about the blood-vessels and were like those described by Sprunt. Others, found in narrow spaces in the pulp substance, resembled more closely the segmented masses observed in the lymph glands and described by Gibson in one of his cases.

[365] It is evident that in the above case we are dealing with two different kinds of calcium and iron deposits. On the one hand, we have a primary deposition of calcium in elastic fibers with a secondary absorption of iron. In these instances, the deposit of calcium is more extensive than that of iron, and many calcified strands can be found in which iron is not demonstrable. Wherever calcium impregnation of the elastic fibers is noted, a preceding process of degeneration can readily be made out.

The other deposit appears to have no relationship to a primary calcification of tissue structures. Here we are dealing with an unusual abundance of iron-containing pigment which lies in the tissue interstices and gradually becomes molded within the spaces. Wherever the neighboring masses incompletely fuse, evidence of apparent segmentation becomes prominent. Thus chains, like threads of large bacilli, are formed. In these structures, the appearance of calcium salts follows the fusion of the iron-containing granules. No calcium was demonstrable in the original granules and it was only seen in those masses which had fused into clear yellow threads.

The material we were dealing with had been fixed in formalin, and to it the criticisms of Hueck might apply. Hueck claimed that the presence of iron in areas of calcification or in bone, as was demonstrated by Gierke, was the result of an artefact; he claimed that either the fixative contained impurities of iron or that the solution of hæmoglobin in the fixative would tend to deposit its iron constituents in the calcified areas. However, under the conditions of the disease here studied (bronzed diabetes), in which enormous quantities of iron-containing pigment were diffusely deposited in the tissues, the opportunity for impregnation of the areas of calcification before death was constantly at hand. Moreover, the precipitation of iron-containing granules, which became closely massed and eventually formed aggregated cylinders, led to a foreign body mass in which the iron constituent was a prominent part.⁴ Under what conditions the calcium salts were attracted to these deposits is not clear, but it was evident that it was a late occurrence.

Our interest in this subject was further aroused by observing the simultaneous deposit of iron and calcium in the arteries.

The calcified plaques of the aorta were examined in five cases [365] with negative results. The peripheral scleroses with calcification (iliac, femoral, tibial, splenic) were also analyzed in 10 cases, and three of these were shown to have a deposit of iron within areas of calcification. These deposits are of interest in indicating that the iron had originated from the blood. Two of the specimens were obtained from elderly individuals (64 and 65 years) having senile gangrene of the foot, and one from a man aged 35 years having diabetic gangrene of the foot and leg.

Advanced processes of calcification, and areas where the degenerative process in the arterial wall acts as an irritant, not uncommonly show the development of a granulation tissue about their periphery. The capillary vascularity of these areas varies greatly, and where the stroma is loose small hæmorrhages are prone to occur. This we observed about the medial calcareous masses in each of the arteries obtained from the cases of senile gangrene. Not only did the border of the calcium deposit show the presence of granulation tissue, but an osteoid structure was also present at various points about the mass. Amidst the capillaries was a loose fibrous stroma in which more or less blood pigment was also demonstrated. In two cases the calcium deposits had, for the most part, involved the media, while lesser masses were found in the intima. In all three, calcified elastic bands were found in the zone between the intima and media, and others showing a similar degeneration were scattered between the muscle fibers of the media. An iron reaction was obtained in the calcareous deposits of the media as well as in the brittle elastic fibers associated with this degeneration. In the areas of advanced calcification the reaction was quite diffuse. The deposits of blood pigment and the neighboring elastic fibers also showed the presence of iron.

The third artery showed plaques of calcification in the thickened intima, while the original lumen was occupied by an organized thrombus. Vascular spaces were still present in the organized mass, while in the more solid areas of fibrous tissue deposits of blood pigment, representing the remains of the former blood clot, were readily recognized. Some of these pigment deposits were close to the calcareous masses. On applica-

[365] tion of the test for iron, not only was a positive reaction demonstrated in the deposits of blood pigment, but a diffuse reaction, more intense in the periphery, was also obtained in the calcareous plaques and in the calcified internal elastic lamina.

In these calcified structures the reaction showed that the iron was present diffusely and in granules. There was no evidence to indicate that the process of calcification had followed the deposition of blood pigment, or that the iron had been obtained from the tissues in which the calcium salts had been precipitated. Many calcified elastic fibers were seen in which iron could not be demonstrated. On the other hand, no iron-containing elastic elements were found in the absence of calcification.

[366] In all of the cases here reported the deposit of iron occurred in calcareous masses under conditions in which there was a local or systemic destruction of blood. In all instances the blood pigment (hæmosiderin) was deposited directly in the tissues, in which subsequently a mixture of iron and calcium salts was found. Similar observations on the impregnation with iron of tissues about old hæmorrhages have been made by S. Ehrlich and others. The presence of iron impregnation, through the agency of blood iron, in areas of previous calcification is in itself not unique. Whether, however, elastic fibers of arteries and organs may, in the absence of calcareous degeneration, absorb iron, as has been suggested by others, was not definitely determined in our material. It is, however, important that the development of pseudomycelial structures through the fusing of masses of blood pigment be recognized and distinguished from infecting micro-organisms. These fungus-like structures receive their calcium component late in their development, and many were found without this element.

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A Mounting Fluid for Gross Specimens in their Natural Colors

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A MOUNTING FLUID FOR GROSS SPECIMENS IN THEIR NATURAL COLORS.*

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Two years ago we reported before this Association a method for fixation and preservation of colors in gross specimens. At that time our attention was concentrated upon the problem of devising a simple means for retaining the natural colors in tissues. We have, we believe, succeeded in this by using a modification of what is known as the Jores' method. The technic of the procedure was published in the Bulletin of this Association in 1915. The method then described had the advantage of being cheap, of ease of application and of giving brilliant color results in vascular tissues. With this fluid for fixation, there was little danger in overdoing the first stage of preserving colors in gross specimens, as is so often the fault of the Kaiserling method. Furthermore, we had the advantage of never losing sight of the color effects which were originally in the tissue and which it was desired to preserve. As you all know, with the Kaiserling method, the color often changes during the initial process and the original color effect must be returned by means of alcohol oxidation.

At the time of our last report we made use of a slightly modified Frost's fluid for permanently preserving the specimens. We used the same ingredients save the omission of sodium fluoride in the solution. Since this time we have studied the problem of the permanent preservation of tissues somewhat more closely. We still find that our primary fixation fluid meets the desires in every respect. We have adhered to the same formula as previously given. The general principle respecting any fluid, used as a fixative, applies to this solution and that is, that the tissue should, if possible, be cut into slabs not over 3 cm. in thickness. The slabs may be of any size in surface area as long as the containers used for their preparation are sufficiently large to permit hardening without folding. Organs of greater thickness may,

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nevertheless, be preserved but require a longer period in the fixing fluid.

We have found several short-comings with Frost's fluid which have led us to abandon it. It not uncommonly occurs that specimens accumulate in the museum preparation room more rapidly than they can properly be taken care of and mounted. Formerly while using the Kaiserling method we carried the specimens through the first two stages to solution number three where a number of specimens would occasionally be allowed to remain until an opportunity was had to mount in the final museum jars.

This we have also done when using Frost's solution. When, however, we returned to our specimens for demonstration, these materials were found extremely disagreeable to handle because of the stickiness of the concentrated sugar solution. The surroundings of the individual employed in mounting specimens in Frost's solution were always in a disagreeable state. The occasional leaking jar on the museum shelf, which under ordinary circumstances is a great annoyance, is doubly so when the leaking fluid contains so much sugar. Lastly the sugar solution has an undesirable effect upon the preserved tissues not unlike that which occurs with glycerin in Kaiserling number three. The tissues have a water-logged appearance and the colors gradually lose their intensity. Another difficulty with the Frost's solution was the occasional moulding and fermentation of the fluid. This is extremely annoying when it occurs in specimens that have been mounted and sealed.

We have found that a preserving fluid containing the same ingredients as the fluid of fixation may be used for the final preservation of the specimens. This fluid is made up according to the following formula:

Carlsbad salts	2.5
Choral hydrate	1.0
Formalin	0.5
Water	100

The Carlsbad salts made up according to the National Formulary has been found very satisfactory. The method has been efficient in our hands, although the oldest mounts are now only about a year and a half old. Still in this time we have found far fewer failures than we have previously had with either the Kaiserling or the Frost's preservative fluid. The colors maintain their intensity and if properly fixed will not leak. The presence of the

small amount of formalin has not shown any harmful effect upon the color of the tissue. The presence of the chloral hydrate appears to neutralize the reducing action of the formalin. There has been an absence of fermentation throughout. Finally the cost of materials is very much less than any of the other preservative fluids that we have tried. It is to be remembered that the successful preservation of gross specimens lies to a great extent in the proper fixation of the tissues prior to the final placing in the preserving fluid. Tissues which continue to leak color must be further fixed before placing them in the final containers and sealed.

On several occasions we have had necessity of returning to our preserved materials for obtaining tissues for microscopic section. We have been struck with the fact that the staining results have been unusually good. In fact, the sections obtained are better than plain formalin fixed tissues. This bears out the finding in other laboratories that formalin fixation is best done with a fluid containing various salts. Five per cent formalin in Ringer's solution gives much better results than the plain formalin dilution.

THE CLASSIFICATION OF STREPTOCOCCI

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THE CLASSIFICATION OF STREPTOCOCCI.*

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Introduction. — In proposing a method of classifying the streptococci, no apology is necessary, since it is generally recognized as of fundamental importance that the members of this group be properly differentiated before any real advance can be made in its study. Almost every investigator has used his own method of classification, depending chiefly upon either morphology, the pathogenicity for certain animals, the power to hemolyze blood, the fermentation complexes, and, in a few instances, upon a combination of certain of these. There is a general lack of correlation, and the majority of these reports are valueless. The hygienist has largely used the fermentation tests, while the obstetrician and medical bacteriologist have observed the hemolytic character, so that the findings of these workers cannot be compared. The same is true of many other investigations. Uniformity of methods, so that correlation may be possible, is the desideratum in the study of streptococci. The marked advance, following proper classification, in our knowledge of other groups, makes it highly desirable that a similar basis may be found in this group.

For the last seven years I have been deeply interested in the study of streptococci, and have had the opportunity of studying large numbers from a great variety of sources. These included strains from general surgical material, blood and throat cultures, materials from the obstetrician, gynecologist, oto-laryngologist, ophthalmologist, and others, strains isolated at autopsies from man and animals, and a variety from milk and other sources. During this time my chief endeavor has been to discover the best method for isolation of these organisms, and the media most favorable for their growth. In many cases, great difficulty was encountered in

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obtaining pure cultures where other organisms were present in the material. In other cases it was sometimes a problem to obtain satisfactory growth, even when the organisms were isolated. In these earlier studies much confusion and irregularity in results were met with from what we have since realized was faulty technic. In these efforts to improve our technic we have tested out a great variety of media and technical methods, as advocated by others. Many of these appeared to be, at the time of their publication, of fundamental importance in our problem, and as offering definite help in the problem of classification. However, it has been our experience, after many careful investigations, that much of this technic, and many of these media, were of little practical use. We have tried growing streptococci in fluid media of varying composition, and have decided that it is more favorable than solid media. The solid media tested included plain agar alone, and with various additions, such as defibrinated blood, serum, and the various carbohydrates. They also included modifications of Loeffler's serum, and the various agar gelatin mixtures. From the experience thus gained we have concluded that the fluid media are the most favorable for the development of streptococci. The advantages of serum broth, which we hold to be the best of these, has been discussed in a former paper. Of the solid media we have found blood agar to be the most useful. It is not advisable in this place to review the literature of the different media, and the various kinds of technic which have been used by others; suffice it to say that many authors have apparently failed to appreciate the fundamental importance of obtaining good growth, and of having convincing evidence of purity in their cultures. It is sometimes extremely difficult to isolate various members of the streptococcus group from each other, and there is strong evidence that the presence of more than one type, in supposedly pure cultures, has led to much confusion. These problems have also been discussed in a former paper. In covering the literature bearing on the use of the hemolytic and carbohydrate tests, I have found that there has been the widest diversion in the methods

employed. For the purpose of general classification, uniformity of methods is fundamental.

Methods of the author. — General: Before considering my method of classification, I will give the media I have found the most useful and the technic I have employed. The collected material is, whenever possible, examined microscopically, and records kept of the types of organisms seen. In all cases where streptococci are found, or in suspicious cases where they are not seen in the direct smear, serum broth cultures are made, and blood agar plates streaked with the material. In certain cases a tube of melted agar may be added to the serum broth for anaërobic cultures. After cultivation blood agar plates are rubbed with a loopful of the serum broth. The surface of the blood agar is covered by as many parallel lines as it is possible to make, in order to thoroughly distribute the material. A streaked plate, such as is illustrated in Hiss and Zinsser's Text-book, is of no value for this purpose. The original blood agar culture serves in a general way to estimate the relative number of the hemolytic and non-hemolytic streptococci and other organisms present.

Isolation from mixed cultures: In certain cases, where the mixture includes such bacteria as *B. proteus* and *B. mucosus capsulatus*, great difficulty may be encountered in obtaining the streptococci. It has been our custom to grow such mixture in dextrose serum broth, and after acid has been produced and the streptococci have fallen to the bottom, to pour off the supernatant fluid and make streak plates on thoroughly dried blood agar. Such plates are not to be used to determine hemolysis, as the blood is somewhat altered by the drying and the reactions are irregular. By this method we are often able to isolate streptococci where other methods fail. Heating the mixture in glass pipettes to 53° C. for twenty to thirty minutes has been shown by Bruecken in our laboratory to check the spreading character

of *B. proteus* and often to destroy it, without its having any apparent effect on the streptococci. Anaërobic plates also help to isolate the streptococci in such cases, without destroying the more sensitive strains.

By the use of serum broth, as I have previously reported, even *B. coli* is considerably outgrown by the streptococci.

In mixed cultures, where pneumococci and streptococci are present, reculturing in serum broth and replating on blood agar is often necessary in obtaining a pure culture. This difficulty is probably due to the sticky character of the pneumococcus. Another method, which finds only a limited application, is to use dextrose serum broth and allow fermentation to continue until the pneumococcus is killed by the acid produced, or the mixture on blood agar may be allowed to remain in the incubator until the pneumococcus succumbs.

There are several other modifications of these methods which are at times useful, such as the use of inulin serum broth to encourage the growth of the pneumococcus, and mannit serum broth for *Streptococcus fecalis*.

Picking of colonies and further growth on differential media: The colonies on blood agar, according to their appearance and reactions, as hemolysis, production of a greening in the colony, size, surface appearance, sticky and non-sticky, watery and dry, and other characters which may be noticeable, are picked to blood agar slants. After growing on the blood agar slants for twenty-four hours, the various carbohydrate serum broth tubes are seeded and incubated for at least a week at 37° C. Daily records are kept of the character and amount of growth, the presence of clouding, precipitation, and the first appearance of acid, if fermentation takes place. Smears are made of a twenty-four hour growth from the lactose serum broth and all the morphological characters are recorded. Similar records are kept of the growth on blood agar.

Media. — The media used are always practically the same and are made as follows :

Blood agar: Plain agar (.6 plus) is sterilized in one hundred cubic centimeter quantities in flasks. A flask is heated in the autoclave and placed in the paraffin oven at 58° C. for some time. It is cooled to 50° C. and five cubic centimeters of defibrinated human blood is thoroughly mixed with the fluid agar. This mixture is poured into petri dishes for blood agar plates, and on the surface of previously prepared agar for blood agar slants, to a depth of about two millimeters. The basic agar for these slants is made of 1.5 per cent agar in normal saline (.85 per cent sodium chloride) filtered, tubed, sterilized, and slanted. This agar base is clear and colorless and makes the whole surface of the finally prepared blood agar slant available for the study of the growth of the bacteria. By this method we have a constant mixture, and comparative readings can be satisfactorily made. It is advisable to add the serum with the red blood cells, as it is an important aid in the production of hemolysin.

Reactions on blood agar: The fact that hemolysis has been shown to be produced at its maximum in serum broth within the first twenty-four hours should be remembered in the reading of blood agar cultures. It apparently takes some time for the hemolysin to diffuse into the surrounding medium, since the maximum hemolysis is not under, but rather over, the twenty-four hours on solid media. If blood is added to the agar when it is too hot, the blood cells become altered and irregular results follow. The same is true if the medium is allowed to become dry. Other conditions, such as the use of media which vary from the isotonic standard, may so affect the red blood cells that hemolysis occurs spontaneously, or under the influence of non-hemolytic strains. Such dissolution of the red cells should not be confused with true hemolysis.

The hemolytic streptococci liberate the hemoglobin, both on solid and in fluid media. Whether there is destruction

of the free hemoglobin is more difficult to determine. In broth the hemoglobin is free, but I have never seen it disappear. It is true it becomes in time changed to methemoglobin. On a blood agar plate, however, the clear zone surrounding the colony appears devoid of any trace of hemoglobin, and if hemolyzed blood be used in the agar, a distinct clear zone develops about the growth. It appears to me probable that the organism grown on ordinary blood agar liberates the hemoglobin through the action of its hemolysin, and then either destroys it or uses it as a food material. The colonies of hemolytic streptococci do not become definitely darker in color, and show no evidence of having in them any of the altered forms of hemoglobin.

The non-hemolytic forms growing on blood agar produce either no hemolysis, or more commonly, a pale bluish green to green, followed by brown, and finally a deep dirty brown, almost black color. These changes in the color of the hemoglobin are probably to be explained as a reduction process.* The bacteria use the oxygen in the medium in which the red cells are embedded and thereby change the osmotic tension, this is followed by the abstraction of oxygen from the oxyhemoglobin, until we have the various stages of the reduction indicated. A curious phenomenon of these green-producing streptococci is the apparent storing of some of the altered hemoglobin in the colony itself. The red blood cells containing this reduced hemoglobin are, I believe, more liable to spontaneous hemolysis. Some of the hemoglobin is certainly liberated and taken into the colony. Apparently it is not entirely destroyed, since the pigment portion of the molecule remains and gives the dark color to the colony. In agar prepared with hemolyzed blood this darkening of the colony is particularly striking, and is in marked contrast to the colony of a hemolytic streptococcus. In this case the blood pigment is free from the cells and is stored in the colony. It is probable that in

* Cole (Jour. Exp. Med., 1914, xx, p. 363) believed that reduction and oxidation play successive rôles in the production of methemoglobin by the pneumococcus. It is most probable that methemoglobin may be produced during the process of reduction.

all of these green producers a certain amount of hemolysis actually occurs, although in the vast majority of them it is not visible. If this were not so, it would be difficult to explain the absence of any clearing on plates with the higher percentages of blood and its presence in the more dilute plates, as Schottmüller and others record. Occasionally, we encounter strains which after longer cultivation (over forty-eight hours) show a narrow, often barely perceptible, ring of clearing close to the colony. This is, of course, a particular type of hemolysis, but is not to be confused with the true hemolysis, and the greening of the colony is usually also present. Smith and Brown described a type (*a*) of hemolysis in which the colonies on blood agar showed a ring with a somewhat greenish discoloration close to the growth, and a second zone surrounding this with no discoloration. In both of these areas partially hemolyzed blood was present. The morphology of the streptococci giving these reactions is quite characteristic for the viridans group, and I believe these strains should be included under the non-hemolytic forms. Mandelbaum and LeBlanc were able to distinguish between the narrow ring of clearing close to the colonies of *Streptococcus mitior* and the hemolytic zone of the *Streptococcus longus* by the fact that in the former the red blood cells beneath the growth are not hemolyzed. In my opinion these types show the extreme of the reduction process with a certain amount of liberation of hemoglobin spontaneously, and are not, in the present state of our knowledge, to be considered as of differential importance.

Ruediger has explained the green coloration on blood agar as due to acid produced by fermentation of carbohydrate substances contained in the serum. This theory he believed was substantiated by the fact that hemolytic streptococci when grown on blood agar containing carbohydrates, fermented by the strain, did not produce hemolysis, but rather greening. His findings I can confirm, as did Anthony and others, but I cannot accept his explanation of the facts. The presence of carbohydrates in media profoundly affects the production or activity of hemolysin. It is difficult to

decide why there is prevention of the hemolysis in these cases. Schlesinger suggested that the acid produced may be the disturbing factor. Sachs believed the failure to produce hemolysin arises from the interference with growth due to acid. It is possible that the acid produced by the streptococci either interferes with the production of hemolysin, or renders it inactive if produced. Acid production is not necessary for the development of the green color, because in the colonies of *B. lactis aërogenes*, an actively fermenting non-hemolyzing organism, green is not produced. *B. bronchisepticus*, a non-fermenting but alkali-producing bacterium, develops a brown coloration in its colonies.

In the case of the hemolytic streptococci, the hemolysin of which is absent or inactive on dextrose blood agar, the organism forms the green coloration in much the same manner as the non-hemolytic green producers. The use of the oxygen of the red-blood cells is indicated when the hemolysin is inhibited.

The non-hemolytic strains which do produce green are not to be clearly distinguished from the strains which are more or less inactive in this respect. These latter have appeared in the literature under such names as *Streptococcus saprophyticus*, and *S. anhemolyticus*. They are found in the same general habitat and same type of case as the more active, definitely green producers, as is indicated in the results of Schottmüller (who originally described *Streptococcus viridans* as varying from fine gray to green or deep-green colonies) as well as of Zangemeister, Hoessli, Le Blanc, Steinert, Rolly, Ruediger, Herrich and Warren, Oille, Graham, and Detweiler, and others. Lyall found that his indifferent strains gave green colonies when grown on blood agar, but when .5 cubic centimeter of an eighteen-hour ascites broth culture was added to one cubic centimeter of a five per cent suspension of washed sheep's red corpuscles, no methemoglobin was produced from the red cells. The conditions under which methemoglobin may be produced are various, as shown by Grüter, and others. In any case, the finding that the factors for methemoglobin production are not present after

eighteen hours' growth in serum broth, is too fine a distinction for purposes of classification.

Carbohydrate media: The preparation of serum broth I have fully described in a former paper. The main points are the following: Take two hundred cubic centimeters of double-strength broth 1.2 plus, add to this one hundred cubic centimeters of water, four grams of the test substance, and four cubic centimeters of Andrade's indicator (decolorized acid fuchsin). This is sterilized in a large flask on three successive days in flowing steam. Beef serum diluted one-half with water is slowly filtered through a Berkefeld filter, and two hundred cubic centimeters added to the above. The whole is then tubed through a sterile funnel into sterile test-tubes, and the tubes incubated for two days to eliminate chance contaminations. Its advantages are that (1) a uniform mixture is obtained in all tubes, (2) there is less liability to contamination than by the addition of the serum with sterile pipettes to each tube, (3) the serum has not been heated, and is, therefore, unaltered, (4) the use of beef serum insures a serum of reasonably constant composition, and is, therefore, useful for comparative tests, (5) these media are not coagulated by the production of acid, and cultural characters can, therefore, be more easily noted.

For anaërobic tests, a tube of melted sugar may be added to this medium after seeding, or the original material may be added to the mixture of serum broth and agar. Careful tests of the serum broth, without the addition of carbohydrates, have failed to produce acid, at least in sufficient quantity to affect the indicator. *B. coli* and other well-known fermenters have been tried, as well as a great variety of streptococci.

Reasons why the titration method is not used: I do not use the titration method to determine the fermentative activity of streptococci for the following reasons: (1) The amount of acid produced is more an indication of the acid point of death, or interference with biological activity, than of actual fermentative power. (2) The vigor of growth so

alters the amount of acid produced that it is liable to wide variation for this reason. Broadhurst has developed this point very clearly in an article on the effect of meat and meat extract media upon the fermentative activity of streptococci. She found quite uniformly that the amount of acid was greater in the meat media, as we would expect, since growth is much better in the infusion than in the plain meat extract media. Thro has concluded that one factor determining the difference in amount of acid in different cultures of the same organism is the variability in the luxuriance of the growth. (3) An arbitrary time limit offers another objection to the method. Three days' incubation, as advocated by almost all the users of the titration method, is often too short to allow fermentation to fully develop. This is true in the case of mannit, which quite often is not fermented until the fourth or fifth day, even in serum broth, and may be longer delayed in plain broth. Lewis had a strain that did not ferment mannit until the seventh day. He probably used plain broth. Titration of such cultures on the third day would give results which would not indicate the biological activity of the organisms. Different sets of the same organism may show variations in the time of fermentation up to and over seventy-two hours. A vigorously growing organism reaches its biological maximum in fewer hours than one less active. (4) The use of arbitrary points of acid production as indicating fermentation, and acid production below these points as not fermentation is not advisable, on the ground of the above reasons, for variation in amounts of acid. Even if streptococci roughly group themselves above or below such an arbitrary point, this is not evidence that we are dealing with differences in the power to or not to ferment the test substances, and may often be explained on the basis of lack of growth, or delayed fermentation. One example from Broadhurst's findings well illustrates this point. A strain of *Streptococcus fecalis*, isolated from the blood in a case of anthrax infection, produced in mannit meat broth 2.3 per cent acid, while only .8 per cent acid was produced in mannit meat extract broth. The latter figure is not considered fermentation

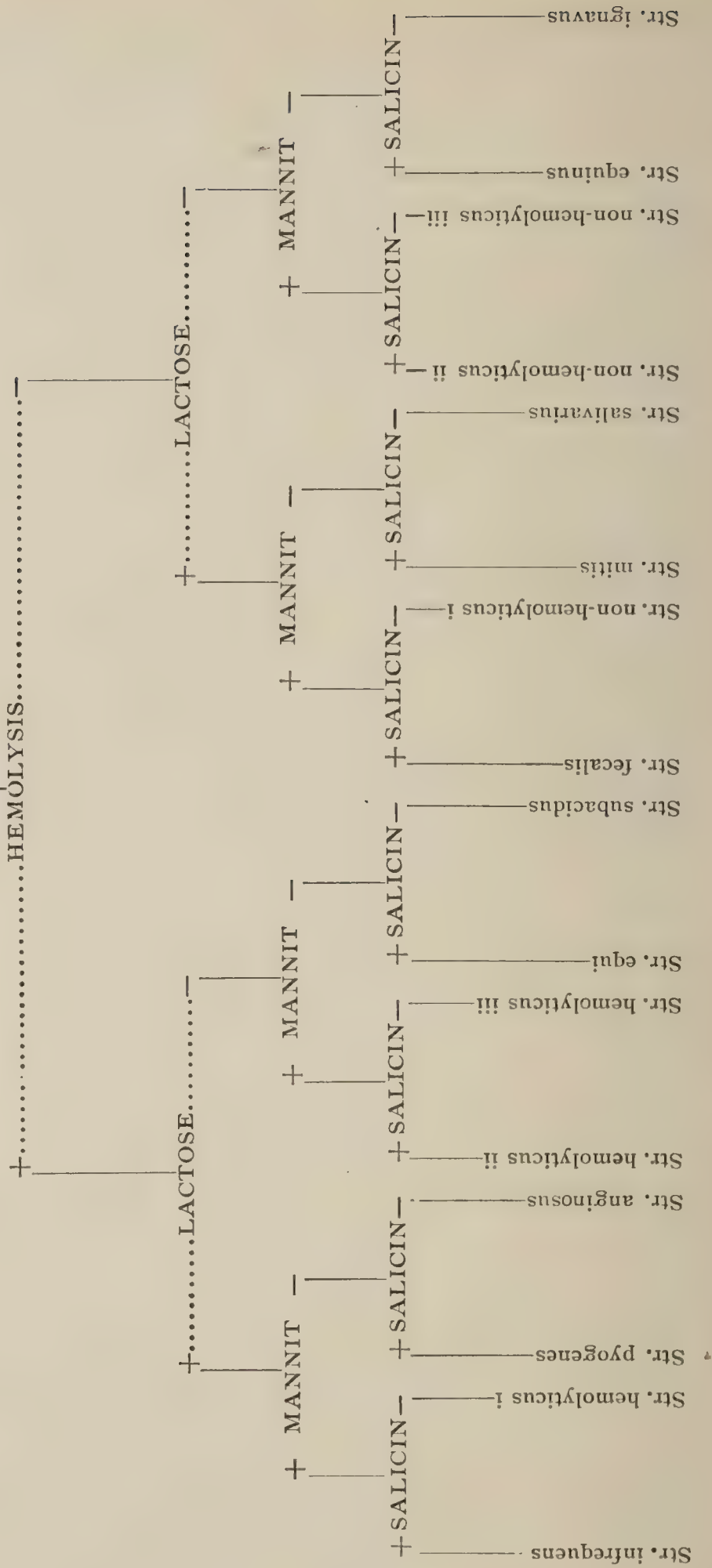
by those using the titration method. (5) The use of control and set titration makes the method too complicated for practical routine application. (6) The personal error in titration will only be a relative one, but, nevertheless, is an objection to the method.

Classification.—The classification which has proved of practical value during several years of routine use, and which I wish here to report, is based on the reactions on blood agar and the fermentation or non-fermentation of the carbohydrates, lactose, mannit, salicin, and inulin in serum broth. The first main division is made by the reactions on blood agar into the hemolytic and non-hemolytic forms. These are then redivided into the lactose and non-lactose fermenters. Each group is again divided into the mannit and non-mannit fermenters, and finally into those that ferment salicin and those that do not.

CHART I.

STREPTOCOCCI.

GRAM POSITIVE COCCI IN CHAINS, NO CAPSULES.



This gives us eight subdivisions under each of the two main divisions, or sixteen groups in all.

The classification of Andrewes and Horder served as a basis, but I have found it necessary to greatly modify it. It was in my hands very difficult to classify organisms according to their scheme, as the same groups of fermentation reactions are found under different headings, and no adequate reason is given for the grouping. Many other workers have reported this same difficulty.

It is some years since I adopted my present method, and new reports on classification that have appeared in the last few years have been found to be easily adapted to this scheme, and the conclusions drawn are equally and often more valuable under my classification than under the various ones used by others.

We have chosen these limited media because they have been shown individually to have some definite significance in dividing the streptococci. Tables I to 17 are made up of all available reports in which the fermentation of the carbohydrates lactose, mannit, salicin, and inulin, as well as the hemolytic test, have been used in distinguishing the organisms. My own results of 1,122 strains are found here. Taking these results together, we are able to bring a relatively large number, 2,463, of streptococci under this classification.

TABLE I.
Showing distribution of the various types of streptococci, classified by author's method.

Source.	Str. Infrequens.	Str. Hemolyticus i.	Str. Pyogenes.	Str. Anginosus.	Str. Hemolyticus ii.	Str. Hemolyticus iii.	Str. Equi.	Str. Subacidus.	Str. Fecalis with Inulin.	Str. Fecalis.	Str. Non-hemolyticus i.	Str. Mitis.	Str. Salivarius.	Str. Non-hemolyticus ii.	Str. Non-hemolyticus iii.	Str. Equinus with Inulin.	Str. Equinus.	Str. Ignavus.	Total.
Nose and throat	48	...	157	54	2	...	4	25	...	6	...	127	155	1	8	28	615
Feces	9	...	4	1	24	...	9	1	4	1	53
Urine and urethra	5	...	3	2	1	9	1	9	...	1	4	3	38
Blood	12	...	67	18	...	1	2	25	4	12	...	29	20	1	5	4	200
Brain and spinal fluid	17	3	1	7	4	2	34
Middle ear	7	...	31	5	3	1	2	2	51
Mastoid	1	45	8	5	3	3	1	66
Joints and synovia	2	1	11	1	1	2	1	20
Uterus and vagina	2	...	3	2	3	7	17
Erysipelas and cellulitis	6	...	24	5	5	...	1	...	1	1	43

Abscesses and pus	9	III	8*	I	2	19	4	21	21†	4	200
Pyorrhea	6	4	2	26	30	5	2	75
Pleural fluid.....	I	10	8	I	6	2	4	4	I	37
Peritoneal fluid	3	2	27	5	2	3	6	I	10	3	I	2	65
Gall bladder.....	I	2	I	I	4	5	14
Compound fracture.....	22	3	I	I	3	I	31
Osteomyelitis and epiphysitis	I	8	2	2	13
Endocarditis	I	3	9	I	5	2	6	12	11	I	3	3	49
Rheumatism	6‡	5	2	3	3	2	21

* Abscess: infraclavicular I, neck 2, parotid I, ovarian I, leg 2, septic finger I.

† Abscesses of tonsil 4, face 3, osteomyelitis of jaw 2, upper lip I, submaxillary I, furunculosis I, abdominal 2, liver I, lumbar I, unknown I, infected incision I, ovarian I, subdural I, pus in otitis media I.

‡ A post-mortem case of chorea gave 4 cultures from blood, pleural, pericardial and peritoneal cavities. Two others came from the pericardium in rheumatism.

Table 1 is arranged to show the frequency of the various types of streptococci from the more common sources listed in Tables 2 to 17.

Discussion of the groups: The hemolytic group: The left side of the chart contains the hemolytic streptococci, there being 1,224 strains, or practically half of the total number. Hemolysis is the distinguishing characteristic. I do not say that hemolysis per se is an indication of virulence or pathogenicity, nor that failure to produce hemolysis indicates non-pathogenicity or saprophytism. What I do firmly believe, however, is that since the vast majority of streptococci, producing severe pyogenic infections, are hemolytic, and that since those failing in this hemolytic character are known to have, as a rule, relatively lower disease-producing powers, we should make use of this important method of differentiation in the classification of these organisms. The criticisms of hemolysis as differential test are largely academic, but any one familiar with the subject appreciates the practical information derived from its use.

Morphology: There are certain morphological characteristics of this hemolytic group which have been so frequently noted in the literature in the descriptions of the pyogenic streptococci that they should receive special notice.

The single cocci in the chains are very frequently not spherical, but rather compressed at right angles to the axis of the chains. The formation of diplococci in the chains is much less accentuated than in the viridans group, and the appearance of large, irregular swollen forms, which have been studied especially by Taddei, are practically only seen among the hemolytic forms.

Ruediger has noted these morphological differences between *Streptococcus pyogenes*, with the appearance in the chains of closely-packed, frequently disc-shaped individual cocci and *Streptococcus viridans*, with elongated, clearly-marked pairs, giving the chains the appearance of having been stretched.

Puppel distinguished between the lancet forms and the "stakett forms" as they occur in milk, and Ernst believed mastitis streptococci could be recognized in the direct smear by this "stakett," or palisade appearance. The true mastitis streptococci are in all probability hemolytic. Heinemann (1915) thought that after animal passages *Streptococcus lacticus* altered its morphology and showed these "stakett" forms. He probably recovered a true hemolytic strain which had invaded the tissues of the animal.

Kocher and Tavel clearly distinguished the streptococci from various sources by their morphology. They state that "Der Streptokokkus brevis, wie man ihn, im Munde und in der Blase findet, hat körnchen die parallel zur Achse abgeplattet sind (längsovale), während die Streptokokken, die man in Blut, in den Organen und im Eiter findet, Körnchen bilden, die senkrecht zur Achse abgeplattet scheinen (querovale)."

v. Lingelsheim (1899) said, "Die Form des Kornes ist beim Streptokokkus longus meist eine runde, daneben kommen aber auch Formen vor, die Abplattungen zeigen und zwar in der Richtung der Längsaxe der Kette. . . . In der queren Axe der Kette zusammengedruckte Formen habe ich dagegen bei dem Streptokokkus longus nicht gesehen," and in Kolle and Wassermann's Handbuch he described the elements of *Streptococcus pyogenes* as approaching round, with slight flattening at right angles to the axis. Smith and Brown noted similar differences between the streptococci with type α hemolysis and those giving type β , that is, the common hemolytic forms.

In much of the earlier literature these morphological characters of streptococci often help us to roughly classify the organisms mentioned. These characters are not absolutely constant, but are so frequently found as to be of definite use.

The majority of writers have practically neglected morphology, or dismiss it by saying it is very variable and unreliable. I have found these morphological differences of great use as corroborative evidence in my classification. It is true, however, that length of chain and size of cocci are extremely variable and unreliable for differentiation.

Distribution: The distribution of the members of this group is much more limited than is that of the non-hemolytic or viridans group. It is largely confined to disease processes in man and animals, and is comparatively rarely found under saprophytic conditions. The more definite sources from which the hemolytic streptococci have been obtained will be seen by reference to the tables.

Pathogenicity: The streptococci in this group have been shown to have powerful invasive qualities, active virulence, setting up violent defensive reactions, together with a high degree of pathogenicity. These organisms are the causative agents in the most severe types of streptococcal disease, such as severe septicemia, erysipelas, peritonitis, and other pyogenic infections. These infections give severe local or general reactions, and are frequently fatal, but even with recovery we have the clinical picture of a severe acute disease.

By the use of the carbohydrate fermentation tests this group is further divided into a number of sub-groups, the leading characteristics of which I will now briefly discuss:

TABLE 2.

Streptococcus infrequens.

Source.	Holman.	Andrewes and Horder.	Ruediger.	Hopkins and Lang.	Smith and Brown.	Kliger.	Broadhurst.	Total.
Throat (scarlet fever)	37	I	38
Blood	4	6	I	I	12
Throat (general)	3	6	9
Human feces	9	9
Tonsilitis	4	3	I	8
Middle ear	I	4	2	7
Erysipelas and cellulitis	I	5	6
Urine	5	5
Throat (dog)	5	5
Infections (various)	3	3
Abscess (retropharyngeal)	2	2
Joints (septic)	I	I	2
Pericardial cavity	2	2
Cervix and blood (sepsis)	2	2
Peritoneal cavity	I	I	2
Throat (cat)	2	2
Pigeon (alim. tract)	2	2
Blood, etc.	2	2
Pus (ethmoid)	I	I
Abscess (cervical)	I	I
Abscess (pelvic)	I	I
Abscess (unknown)	I	I

TABLE 2. — *Continued.*

Source.	Holman.	Andrewes and Horder.	Ruediger.	Hopkins and Lang.	Smith and Brown.	Kliger.	Broadhurst.	Total.
Gland (scarlet fever).....	I	I
Gland (adenitis)	I	I
Thrombus	I	I
Acute epiphysitis	I	I
Appendicitis	I	I
Conjunctivitis	I	I
Frontal sinus.....	I	I
Throat (measles).....	I	I
Pleural fluid	I	I
Guinea-pig (abscess).....	I	I
Total	29	12	63	3	I	4	20	132

Streptococcus infrequens (Table 2) is represented by one hundred and thirty-two strains. It is a hemolytic streptococcus which ferments lactose, mannit, and salicin. I consider that this group is sufficiently numerous and important to receive a name, and I have therefore applied the above nomenclature to indicate its relative infrequency. Ruediger has given particular interest to this streptococcus by finding it so often in throat conditions, more especially in scarlatinal angina, thirty-seven of his sixty strains from scarlatinal throats falling into this group. He also found it four times in otitis media, twice in pericardial fluid, once in suppurative adenitis, and once in conjunctivitis from persons with scarlet fever. Likewise, Andrewes and Horder found it once in a gland, Kliger once in the throat, and I have recovered it

once from the heart's blood in a fatal case of scarlet fever. The twelve tabulated strains from the blood include five from puerperal septicemia, three from general sepsis, and one each from endocarditis, sepsis with purpura, and scarlet fever. One strain was recovered from a thrombosed vein. The strains of hemolytic streptococci with mannit fermentation, reported by Henke and Reiter, from abscesses of the tonsil, and those of Davis, from the crypts of the tonsils in chronic arthritis cases, probably belong here.

I have grown *Streptococcus infrequens* from the tonsil of a woman suffering with chronic arthritis. The clinical symptoms improved following removal of the tonsils. An autogenous vaccine of this organism was used, and it appeared to be helpful. In a case of chronic cystitis, I have recovered, on three different occasions, at intervals of six and sixty-three days, this streptococcus from the urine. An autogenous vaccine was administered and the condition gradually cleared up. This organism also was grown from the blood of a student with endocarditis. It grew in the nipple, like colonies so characteristic of hemolytic streptococci, showing a distinct and sharp hemolysis, and in the serum broth media gave a peculiar yellow precipitate with a fluorescence in the supernatant fluid. This latter peculiarity has been observed in several hemolytic strains of different types. Somewhat similar reactions have been reported by Pricolo, Jupille and Krumwiede, and Valentine.

Two of these streptococci, retested after two hundred and fifty-seven and three hundred and twenty days, showed the same biological characters as when freshly isolated.

TABLE 3.
Streptococcus hemolyticus i.

Source.	Holman.	Floyd and Wolbach.	Total.
Appendix.....	I	I	2
Mastoid	I	I
Joint	I	I
Autopsy	I	I
Total.....	3	2	5

Streptococcus hemolyticus i (Table 3), with only five members, is too uncommon to merit a name. It gives hemolysis and fermentation of lactose and mannit without attacking salicin. I have only found it three times.

TABLE 4.
Streptococcus pyogenes.

TABLE 4. — *Continued.*

Source.	Holman.	Andrewes and Horder.	Ruediger.	Leutscher.	Hopkins and Lang.	Floyd and Wolbach.	North, White, and Avery.	Smith and Brown.	Kliger.	Broadhurst.	Total.
Blood (puerperal)	2	2	I	5
Human feces	4	4
Milk	4	4
Uterus	3	3
Eye	3	3
Urine and urethra	2	I	3
Spleen autopsy	2	2
Cyst of spleen	I	I
Gangrene of leg	I	I
Guinea-pigs *	120	120
Dog (throat)	7	7
Dog (esophagus and stomach)	21	21
Dog (intestine)	9	9
Blood, etc.	2	2
Total	485	68	61	I	47	4	I	34	22	39	762

* Guinea-pigs: heart's blood 43, pleural cavity 38, peritoneal cavity 18, uterus 10, abscess of mamma 3, lung 2, trachea 2, urine 2, abscess I, vagina I.

Streptococcus pyogenes (Table 4) is the largest group, with seven hundred and sixty-two strains. It ferments lactose and salicin, and hemolyzes blood. The name indicates its pyogenic character. It is the common streptococcus found in purulent conditions, where the streptococcus is the

etiological factor (III being from pus and abscesses, 76 from infection of the middle ear and mastoid, 46 from serous cavities, 17 from brain or cerebrospinal fluid, 8 from osteomyelitis, 11 from joints, and 10 other purulent conditions, giving 273 strains from sources where macroscopic pus is the common finding).

It is also the organism most frequently recovered from the blood in the severe streptococcemia. The one hundred and twenty strains from guinea-pigs were practically all grown from animals with severe spontaneous infections. In fact, it can be said that the great majority of the strains which I have studied and grouped under this heading, with the exception of some of the throat strains, were derived from infections of a more or less severe nature. The reactions are constant. I have retested a number of strains after prolonged cultivation, and have not found any alterations in the biological characters. These tests have been made on different strains at intervals of two hundred and ninety-eight, three hundred and three, and three hundred and ninety-two days. One strain was tested after three hundred and ninety-two days, without being transferred during that period, and, nevertheless, showed the same characters as in the original test.

TABLE 5. — *Continued.*

Source.	Holman.	Andrewes and Horder.	Leutscher.	Hopkins and Lang.	Floyd and Wolbach.	Smith and Brown.	Kliger.	Broadhurst.	Total.
Blood (rheumatism)	2	2
Blood (puerperal)	2	2
Urine	2	2
Vagina	I	I
Uterus.....	I	I
Abortion.....	I	I
Septic finger	I	I
Joint	I	I
Gall bladder	I	I
Dog (intestinal tract)	2	2
Blood, etc.....	3	3
Total.....	66	4I	7	6	3I	8	2	5	166

Streptococcus anginosus (Table 5) I have named from Andrewes and Horder's classification. They are, however, very indefinite about the hemolytic character of the members of their group. This organism hemolyzes blood and ferments lactose, but does not attack mannit, salicin, and inulin. It is represented by one hundred and sixty-six strains, fifty-nine of which came from the nose and throat. Of these, twenty-six came from scarlatinal, measles, and diphtheria throats, ten from tonsilitis, and four from membranous stomatitis. Where these organisms are found in the blood stream or tissues, the probability of their invading from the throat cavity should not be overlooked. The nine strains grouped under

endocarditis in Table I include six from malignant endocarditis, one endocarditis, and two from the valves of the heart at autopsy. Of the eight listed under abscesses, four came from regions strongly suggesting their derivation from the throat. It should be noted, in the support of Andrewes and Horder's suggestion, that some relation exists between pus formation and salicin fermentation, that there are remarkably few of these strains which can definitely be classed as the causative agent in purulent conditions. The eighteen strains from blood in Table I include eleven from sepsis (3 were puerperal and 1 from meningitis, while in the others the type of sepsis is not given), seven from heart's blood at autopsy (2 from rheumatism, 2 endocarditis, and 1 scarlet fever). The seven strains from milk, as well as the one strain from a throat, reported by Smith and Brown, in a study of epidemic sore throat, and the five strains of Leutscher's from epidemic sore-throat cases, are of interest. It will be noted, however, that Smith and Brown found seventeen strains of *Streptococcus pyogenes* in the throat, one in the blood, one in peritoneal pus, four in milk, and eleven among other strains studied by them from various epidemics. These authors do not consider that the same organism is responsible in all the epidemics, and in this I agree. There is no doubt that it has been confused, by early workers, with the non-hemolytic streptococcus *salivarius*, chiefly on account of the very long chains found in the latter.

TABLE 6.
Streptococcus hemolyticus ii.

Source.	Holman.	Smith and Brown.	Total.
Throat	2	2
Peritoneal cavity.....	2	2
Abscess	I	I
Total.....	I	4	5

Streptococcus hemolyticus ii (Table 6) is only represented by five strains. It is a hemolytic non-lactose, non-inulin fermenting streptococcus, which ferments mannit and salicin. Smith and Brown have given this small group unusual prominence. They described two strains from the peritoneum of operated cases, and two from the throats of associates of these patients, in an outbreak of epidemic sore throat. Agglutination tests confirmed the differentiation of these streptococci. Our own strain was from an abscess of the chest wall.

TABLE 7.
Streptococcus hemolyticus iii.

Source.	Holman.	Total.
Empyema	I	I
Blood.....	I	I
Total	2	2

Streptococcus hemolyticus iii (Table 7) has only two representatives. It is hemolytic and only ferments mannit. I have found it twice, once in empyema, and once from the blood in puerperal sepsis.

TABLE 8.
Streptococcus equi.

Source.	Holman.	Andrewes and Horder.	Hopkins and Lang.	Floyd and Wolbach.	Smith and Brown.	Kliger.	Total.
Throat	2	I	I	4
Blood	I	I	2
Abscess	2	2
Gall bladder.....	I	I	2
Endocarditis	I	I	2
Brain	I	I
Blood (rheumatism).....	I	I
Pericardium (rheumatism)	I	I
Gland	I	I
Joint.....	I	I
Compound fracture.....	I	I
Urethra	I	I
Guinea-pig (gland)	I	I
Total.....	9	6	I	2	I	I	20

Streptococcus equi (Table 8) has been so named following the description of that organism by Bemelmans and others. This streptococcus ferments salicin and is hemolytic. It is represented by twenty strains. Laabs (1910) reported it as hemolytic. Pricolo (1910) described this organism as hemolytic and as never coagulating milk. He tested it on lactose, mannit, salicin, inulin, and levulose, but only said that no gas was formed, the reaction was not given. Bemelmans (1913) gave the fermentation reactions as follows,

dextrose, saccharose, and salicin were fermented, while lactose, mannit, and inulin were not. Koch and Pokschischewsky (1913) found *Streptococcus equi* strongly hemolytic, it did not ferment mannit, and six out of ten strains did not attack lactose. Maas found that all his ten strains of streptococci from the horse fermented salicin, four did not ferment lactose, three attacked mannit, and four did not. He was certainly dealing with a variety of strains, including *Streptococcus equi*. Gminder reported a hemolytic non-lactose fermenting strain from horse sputum, which probably belongs here. This streptococcus has received considerable attention in being isolated from cases of sore throat in horses, spoken of as gourme or strangles. It is, of course, true that *Streptococcus pyogenes* is also responsible for many infections in the horse, as it is in many other animals. It is very questionable whether a true capsule is formed by *Streptococcus equi*, and I prefer to consider it as non-encapsulated.

The sources from which it was obtained are very variable, and it is relatively unimportant in human disease. However, we cannot draw too close a line between strains that are pathogenic for animals and those pathogenic for man. Kliger reported a streptococcus with the characters of this type from the blood in sepsis, and I have encountered it once from the heart's blood at autopsy. I have also recovered it from two abscesses, one in the thigh, and one post-auricular. Smith and Brown found it once in the throat. Agglutination tests of this strain were unsatisfactory, owing to spontaneous agglutination. One strain which I isolated from a compound fracture gave the same reaction on blood agar and in the carbohydrate tests, after having been stored for four hundred and sixty days on blood agar.

TABLE 9.
Streptococcus subacidus.

Source.	Holman.	Andrewes and Horder.	Hopkins and Lang.	Floyd and Wolbach.	Broadhurst.	Total.
Blood.....	1	1	12	14
Abscess	5	1	1	7	14
Blood (scarlet fever).....	2	9	11
Throat	8	8
Throat (scarlet fever)	3	5	8
Throat (diphtheria).....	6	6
Scarlet fever (various)	6	6
Pleural cavity	2	4	6
Mastoid	2	3	5
Scarlet fever (autopsy)	5	5
Endocarditis	5	5
Cellulitis and erysipelas	1	4	5
Septic wounds, etc.	5	5
Brain and spinal fluid	4	4
Gland.....	2	2	4
Uterus (septic).....	3	3
Spinal fluid (scarlet fever)	3	3
Tonsils...	3	3
Middle ear	1	2	3
Peritoneal cavity.....	1	1	1	3
Joint	2	2
Unknown	2	2

TABLE 9. — *Continued.*

Source.	Holman.	Andrewes and Horder.	Hopkins and Lang.	Floyd and Wolbach.	Broadhurst.	Total.
Antrum	I	I
Pericardial cavity	I	I
Miscarriage	I	I
Eczema	I	I
Gall bladder	I	I
Blood, etc.	I	I
Total	28	7	I	94	I	131

Streptococcus subacidus (Table 9) is the name I have given to this group of hemolytic streptococci which shows no fermentation with the four carbohydrates. They practically all ferment either saccharose or dextrose, and are, therefore, not entirely devoid of fermentative ability. There are one hundred and thirty-one strains in this group. Floyd and Wolbach found an unusually large number (94) in their series, and I am inclined to believe that they are not as common as this would indicate, and the conclusion is forced upon me that the fault lay in their media, their indicator, or some other failure in their technic. Moreover, the sources would suggest that the majority of their strains were highly pathogenic, and it is probable that they were sensitive to unfavorable conditions in artificial media, even if such media were satisfactory for other streptococci. Krumwiede and Valentine's findings, that the serum water media are not favorable for fermentation tests, are in keeping with my results in testing out the various fluid media. That there are

streptococci with this limited fermenting power I have no doubt, but I consider them quite uncommon. My own twenty-eight strains are from a variety of sources, and are definitely different from the other hemolytic streptococci in their lower fermentative power. They all gave fermentation of either dextrose or saccharose.

Streptococcus viridans group: On the other half of the chart are grouped the non-hemolytic streptococci. These streptococci frequently develop a bluish green, green, or dark green to brown color, but there are many that show little or no change on the blood agar. The color appears to be mostly in the colony, but some is also in the medium below and close to it. The non-hemolytic streptococci here grouped include those appearing in the literature as *Streptococcus viridans*, *Streptococcus anhemolyticus*, *Streptococcus saprophyticus*, *Streptococcus lacticus*, and under many other names. Similarly the name *parapneumococcus* was suggested by Natwig, but I believe that the name *Streptococcus viridans*, despite the fact that the green coloration is not always seen on blood agar, should be retained for the group, since it has been so long in use. There is not sufficient evidence at present for dividing the strains with no hemolysis and no green production from those developing green on blood agar, as is discussed above.

Morphology: The morphology of the *viridans* group is somewhat varied among the different members, but there are some general characteristics that serve to distinguish it from the hemolytic group. The cocci are usually arranged in elongated pairs, which are distinctly separated from the next pair, and give the chain a loose appearance, as if it had been stretched, as described by Ruediger. This arrangement in elongated pairs is common to the group. Certain members, however, show other distinguishing features. The *Streptococcus salivarius* is characterized by its growth in long chains, often extremely long, which is further indicated by the macroscopic appearance of a loose, fluffy precipitate in

the serum broth cultures. This character of the precipitate is usually in marked contrast to the finer, more granular one, seen with the other members of the group. The morphology of these non-hemolytic streptococci has already been considered, under the discussion of the hemolytic group. The illustrations given by Kocher and Tavel of streptococci from the vagina and the mouth, and the many plates shown by Gordon of streptococci from saliva demonstrate these morphological characters. It is also not uncommon to find bacillary forms in the chains. This was noted by Gordon in his early work on *Streptococcus scarlatinæ*, and at first thought by him to be a specific character. Bacillary forms are to be noted in most of the members of this group, under certain undetermined conditions of culture, of which the age seems to be important, and suggests that the organism is still able to grow, but is losing its ability to reproduce.

Distribution: The members of this group are very widely distributed, being found in the alimentary tract of man and animals, in the air, water, and almost always in milk, as well as from a variety of other places. They are also found in different parts of the body of man and animals in conditions of disease and lowered resistance. They are, as a rule, much more resistant to harmful influences, which accounts for their common occurrence under saprophytic conditions.

Pathogenicity: These streptococci are sharply differentiated from the hemolytic strains, not only by their failure to produce hemolysis, but by the character of the diseased conditions which they bring about. Macroscopic pus is but rarely produced, either in the natural disease or in experimental animals. Members of this group are the common cause of the chronic streptococcal infections. They have high invasive power, and attack tissues in a state of lowered resistance, stimulate little reaction on the part of the body, apparently render the tissues more susceptible to reinfection, and death results only after a prolonged course or repeated infections. Clinically, these cases are characterized by a

mild and chronic course, with frequent exacerbations. True it is that the final stages may be very severe, and it is not uncommon to find members of the hemolytic group invading and increasing the severity of the condition.

Having first determined the non-hemolytic nature distinguishing the members of this group, we proceed to divide them into their various sub-groups by means of the carbohydrates, as was done for the hemolytic group. This has been done in our chart, and I shall now discuss the individual sub-groups.

TABLE 10.
Streptococcus fecalis.

Source.	Holman.	Andrewes and Horder.	Hopkins and Lang.	Kliger.	Hartzell and Henrici.	Broadhurst.	Total.
Human feces.....	14	3	2	5	24
Milk	2	2	12	16
Urine and urethra.....	3	3	2	1	9
Unknown	2	1	6	9
Blood	2	4	1	7
Peritoneal cavity.....	6	6
Throat	5	1	6
Blood (endocarditis)	2	1	2	5
Water	5	5
Abdominal wall.....	3	3
Abscess (ischiorectal, psoas, and leg).....	3	3
Osteomyelitis.....	2	2
Pericardial cavity	1	1	2
Pleural cavity	2	2

TABLE 10. — *Continued.*

Source.	Holman.	Andrewes and Horder.	Hopkins and Lang.	Kliger.	Hartzell and Henrici.	Broadhurst.	Total.
Ear	I	I	2
Pyorrhea	I	I	2
Rheumatic lesion	I	I
Infarct of spleen (endocarditis)	I	I
Stump of leg.....	I	I
Burn on buttocks	I	I
Pus from hand	I	I
Incision in breast	I	I
Compound fracture.....	I	I
Cellulitis (arm).....	I	I
Gall bladder.....	I	I
Diplococcus rheumaticus (Beattie)	I	I
Guinea-pigs *.....	48	48
Dog (alimentary tract †).....	75	75
Cat (alimentary tract ‡).....	45	45
Pigeon (alimentary tract)	4	4
Hen (alimentary tract)	3	I	4
Frog (subcutaneous tissue).....	I	I
Horse feces	I	I
Blood, etc.	7	7
Total	107	11	8	9	I	162	298

* Guinea-pigs: peritoneal cavity 26, heart's blood 10, throat 5, intestine 2, mammary abscess 2, uterus 2, vagina 1.

† Dog: throat 35, esophagus and stomach 8, intestine 13, feces 19.

‡ Cat: throat 22, esophagus and stomach 8, intestine 15.

TABLE 10. — *Concluded.*
Streptococcus fecalis.
(Variety with inulin.)

Source.	Holman.	Andrewes and Horder.	Hopkins and Lang.	Broadhurst.	Total.
Blood (endocarditis)	2	2	4
Human feces.....	1	1
Guinea-pig (peritoneal cavity)	9	9
Guinea-pig (intestines)	4	4
Guinea-pig (uterus).....	4	4
Guinea-pig (heart's blood)	3	3
Guinea-pig (pleural cavity).....	2	2
Guinea-pig (throat)	2	2
Milk	1	1
Cat (throat)	2	2
Cat (esophagus and stomach)	5	5
Dog (throat).....	21	21
Dog (esophagus and stomach).....	16	16
Hen (stomach).....	2	2
Horse feces	1	1
Blood, etc.....	13	13
Total.....	25	2	2	61	90

Streptococcus fecalis (Table 10), one of the most important of these organisms, was named from Andrewes and Horder, and is represented by two hundred and ninety-eight strains. There is no hemolysis, and fermentation is positive for lactose, mannit, and salicin. Inulin is not fermented. Over half, or one hundred and sixty strains, were grown from feces, or the alimentary tract of man or animals. Of these alimentary strains, six came from the human throat, and sixty-five from the throat of dogs (35), cats (22), guinea-pigs (5), and hens (3). It is not surprising to find streptococci of fecal origin in the throat cavity of these animals. Many of the other sources of origin for these organisms strongly suggest fecal contamination or invasion from the intestines, as twenty-six strains from the peritoneal cavity of untreated or spontaneously dead guinea-pigs, nine from human urine and the urethra, six from the human peritoneal cavity, and others as seen in the table.

Streptococci, with the characters of this group, are among those associated with rheumatism and endocarditis, six in the table were from endocarditis, and three from rheumatic lesions. The streptococci isolated by Rosenow from cholecystitis which fermented lactose, mannit, and salicin, apparently belong here, although the reaction on inulin is not given. The three strains, with mannit and salicin fermentation, reported by Lyall, from rheumatism, should, undoubtedly, be placed in this group, if we grant lactose fermentation as positive. Rosenow's strains from rheumatism, with mannit fermentation, are probably also *Streptococcus fecalis*, although some of them may fall under the non-hemolytic groups, i, ii, or iii. Many more strains, giving the characteristic carbohydrate reactions, but in which the hemolytic test was not made, are very probably of the *Streptococcus fecalis* type. Such undetermined types are mentioned by Martin (1907), Horder 1906 (the "diplococcus rheumaticus" strains ii and iii), Beatti and Yates (1911), and many others. Besides these, many strains from feces, which have been reported with only the fermentation tests given, may be included here, owing to the relative infrequency of hemolytic streptococci

from this source. The great majority of those giving the fermentation complex for this type came from human feces. That mannit fermentation has developed, in certain of the strains supposedly transmuted through animal passage, by Rosenow and Heinemann, suggests the possibility of secondary invasion or contamination. The forty-eight strains which I obtained from guinea-pigs, I am convinced, were invading forms from the bowel. The *Streptococcus fecalis* is, as a rule, particularly resistant to a variety of adverse conditions. As previously reported, I have dried such an organism for one hundred and seventy-four days on cover-glasses, and found it unaltered in its reactions and characters. I have allowed it to remain in sealed blood agar tubes for four hundred and forty-eight days without transfer, and on reculturing, found it unaltered. One strain was heated for six hours, at 58° C., and another for six hours, at 60° C., and twenty-three hours at 58° C., without altering their reactions when grown and retested. Houston found that fecal streptococci, giving the carbohydrate reactions for this group, showed, after twenty days in normal saline, the highest percentage of viable forms, while in sterile tap water they remained alive longer (51 days) than any other of the strains tested.

The variety with inulin, numbering ninety, are largely from the same general sources, seventy-one coming directly from animals. This variety is uncommon among human streptococci. Four of the strains, however, were obtained from endocarditis cases in man.

TABLE II.
Streptococcus non-hemolyticus i.

Source.	Andrewes and Horder.	Floyd and Wolbach.	Broadhurst.	Total.
Cat (throat)	3	3
Urethra	I	I
Ear	I	I
Appendix	I	I
Unknown	I	I
Total	2	I	4	7

Streptococcus non-hemolyticus i (Table II), which does not hemolyze, and ferments lactose and mannit, but not salicin nor inulin, with seven members, is too rare an organism to merit a special name. I have not found this streptococcus in my work. Others, however, have reported such forms.

TABLE 12.
Streptococcus mitis.

TABLE 12. — *Continued.*

Source.	Holman.	Andrewes and Horder.	Hopkins and Lang.	Floyd and Wolbach.	North, White, and Avery.	Smith and Brown.	Kliger.	Hartzell and Henrici.	Broadhurst.	Total.
Autopsy.....	I	I
Middle ear	I	I
Blood (rheumatism)	I	I
Throat (measles).....	I	I
Erysipelas	I	I
Strept. rheumaticus	I	I
Diploc. rheumaticus (Poyn- ton)	I	I
Guinea-pig*	18	18
Cat†	60	60
Dog‡	30	30
Horse feces.....	I	3	4
Blood, etc.....	4	4
Total	171	34	10	12	10	9	16	14	119	395

* Guinea-pig: heart's blood 9, peritoneal cavity 3, pleural cavity 3, uterus 1, vagina 1, abscess 1.

† Cat: throat 5, esophagus 11, intestine 37, feces 7.

‡ Dog: throat 6, esophagus 8, intestine 11, feces 5.

Streptococcus mitis (Table 12), represented by three hundred and ninety-five strains, is the most common of the non-hemolytic strains. It ferments lactose and salicin, but not mannit nor inulin, and fails to hemolyze blood. The name is adopted from Andrewes and Horder. The sources indicate a wide distribution, one hundred and twenty-seven from the human nose and throat, twenty-six from pyorrhea

twenty-one from general abscesses, and twenty-nine from the blood. The twenty-one general abscesses, to which may be added four appendix abscesses, had also, as a rule, other organisms present. Three appendix abscesses and three ischio-rectal abscesses were mixed with *B. coli*, two skin infections with *Staphylococcus pyogenes aureus*, one thigh abscess with *Streptococcus pyogenes* and *B. mucosus capsulatus*, and one retropharyngeal abscess with *B. acidi lactici*. Similar mixtures were found in many other infected sources, as in the pus from an ear *Streptococcus pyogenes* and *B. proteus* were in association with *Streptococcus mitis*. The twenty-nine strains from blood include ten which I have studied (2 from heart valves in endocarditis, 3 from heart's blood at autopsy, 3 from blood cultures in sepsis, 1 from venous thrombosis, 1 from a blood culture in rheumatism), five from Kliger (2 sepsis, 1 puerperal sepsis, and 2 endocarditis), three from Hopkins and Lang (1 sepsis and 2 endocarditis), and eleven from Andrewes and Horder (6 malignant endocarditis at autopsy, 1 blood culture in malignant endocarditis, 2 terminal septicemia, with duodenal ulcer, 1 with tuberculosis and fractured jaw, and 1 with anthrax infection). I am not convinced that Andrewes and Horder actually used the hemolytic test in all of their cultures. In this group we also have fourteen strains from rheumatic and endocarditis cases. It is impossible to tabulate the strains which have been grouped by the fermentation complexes alone, although many streptococci are there found which probably belong to this group.

A variety with inulin has not been included on account of the impossibility of separating the pneumococcus, as listed in the tables of others, from streptococci giving these fermentations. It has been my finding that almost all organisms showing these reactions, including inulin, are pneumococci. The classification of a variety of *Streptococcus mitis* with inulin can only be admitted when the classical characters of the pneumococcus are absent, *e.g.*, typical bullet-shaped morphology, demonstration of capsules in appropriate media by stains or with India ink, and characteristic watery colonies with greening on blood agar. The pneumococcus colony is

fairly large, showing a dimple in the center, due to the falling in of the watery colony after it has reached its maximum. I believe that the inulin variety of *Streptococcus mitis* exists, and it may be more common than I have indicated.

TABLE 13.
Streptococcus salivarius.

Source.	Holman.	Andrewes and Horder.	Hopkins and Lang.	Floyd and Wolbach.	North, White, and Avery.	Smith and Brown.	Kliger.	Hartzell and Henrici.	Broadhurst.	Total.
Throat	58	8	1	7	9	5	16	104
Pyorrhœa	18	2	10	30
Milk	6	10	9	4	29
Tonsils	12	3	4	19
Nose	14	14
Blood (endocarditis)	3	3	5	11
Throat (diphtheria)	10	10
Throat (scarlet fever),	7	1	8
Blood	4	2	1	7
Pneumonia	5	1	6
Gall bladder	5	5
Abscess (face and lip)	4	4
Pleural cavity	4	4
Pericardial cavity	4	4
Mastoid	2	1	3
Cellulitis	2	1	3
Unknown	1	2	3
Osteomyelitis (jaw)	2	2
Middle ear	1	1	2

TABLE 13. — *Continued.*

Source.	Holman.	Andrewes and Horder.	Hopkins and Lang.	Floyd and Wolbach.	North, White, and Avery.	Smith and Brown.	Kliger.	Hartzell and Henrici.	Broadhurst.	Total.
Eye	I	I	2
Peritoneal cavity	2	2
Abdominal abscess	2	2
General abscess	I	I	2
Liver abscess	I	I
Lumbar abscess	I	I
Ovarian abscess	I	I
Subdural abscess.....	I	I
Submaxillary abscess.....	I	I
Ear	I	I
Blood (rheumatism)	I	I
Rheumatism	I	I
Infected incision	I	I
Medulla of bone	I	I
Joint	I	I
Furunculosis.....	I	I
Feces	I	I
Chancroid	I	I
Cat*.....	2	2
Dog †	8	8
Blood, etc.	9	9
Total	146	42	13	33	18	5	3	10	39	309

* Cat: esophagus I, intestine I.

† Dog: throat I, esophagus, etc., 6, feces I.

Streptococcus salivarius (Table 13) is a name adopted from Andrewes and Horder. It is characterized by the fermentation of lactose, no fermentation of mannit, salicin nor inulin, and no hemolysis of blood. The group includes three hundred and nine strains, half, or one hundred and fifty-five coming from the nose and mouth. The number associated with pus formation, fifty-one, is small and when we exclude thirty strains from pyorrhea, four from abscesses of the face and lip, four from abscesses of tonsil, two from osteomyelitis of the jaw, one from a submaxillary, and one from a subdural abscess, where the possible invasion from the mouth is evident, we have but nine strains. This is in keeping with the suggestion of Andrewes and Horder that there is some relation between salicin fermentation and pus formation. Moreover, in most of these cases, other bacteria were also found in the pus. Thirteen strains of this group were associated with rheumatism and endocarditis. The twenty strains from blood include ten from endocarditis. Of my own eight strains, three were from blood culture in endocarditis, one in sepsis, one in rheumatism, two from heart's blood at autopsy, and one from a thrombosed vein. Floyd and Wolbach had one from the heart's blood. Hopkins and Lang had five from the blood in endocarditis and four in septicemia (1 following pneumonia, 1 a ruptured aorta, 1 empyema and peritonitis, and 1 suppurative arthritis). A series of retests have been made with some of these streptococci. Single strains from throat, endocarditis, nose, sore throat, and abscess of upper lip were retested after one hundred and two, one hundred and thirty-five, two hundred and thirteen, three hundred and ninety-four, and four hundred and eighty-eight days, respectively, and did not show any alteration in their characters. An apparently pure culture of *Streptococcus mitis* from a throat which had shown extremely long chains of pairs (an uncommon finding for *Streptococcus mitis*) in the original test was retested after four hundred and nineteen days. It then showed chains of several hundred units, arranged in distinct pairs, and refused to ferment salicin. There is no doubt in my mind that this culture was originally

a mixture of *Streptococcus mitis* and *Streptococcus salivarius*, and that the latter survived after the long storage on media. Lyall reported one strain (granting the fermentation of lactose) from rheumatism, and one from endocarditis. Horder had five strains from rheumatism and seven from malignant endocarditis, and Gordon two from endocarditis. These last two authors did not use the hemolytic test and some of their strains may have been *Streptococcus anginosus*.

Here, as in the last group, the variety with inulin is not considered, as certain pneumococci do not ferment salicin, and the remarks under *Streptococcus mitis* are equally applicable here.

TABLE 14.

Streptococcus non-hemolyticus ii.

Source.	Holman.	Andrewes and Horder.	Hopkins and Lang.	Broadhurst.	Total.
Dog (throat).....	4	4
Guinea-pig (intestines)	3	3
Urine	I	I
Blood (endocarditis)	I	I
Blood, etc.	I	I
Total.....	3	I	I	5	10

Variety with inulin.

Blood, etc.	I	I
Total.....	I	I

Streptococcus non-hemolyticus ii (Table 14), with mannit and salicin fermentation and no hemolytic power, has ten strains. I have found this organism in the intestinal tract of guinea-pigs, but never from any other source. After a large

peritoneal injection of this organism into a guinea-pig, a similar organism was recovered from the peritoneum and heart's blood. The animal was killed after forty-eight hours. Broadhurst found four strains in a dog's throat. Hopkins and Lang had one strain from the blood in human endocarditis.

TABLE 15.

Streptococcus non-hemolyticus iii.

Source.	Floyd and Wolbach.	Broadhurst.	Total.
Unknown.....	I	I
Dog feces.....	I	I
Total.....	I	I	2

Variety with inulin.

Hen (stomach).....	2	2
Total.....	2	2

Streptococcus non-hemolyticus iii (Table 15) with mannitol fermentation has not been found in my work. Floyd and Wolbach report one from an unknown source and Broadhurst one from dog's feces. The variety with inulin is represented by two strains from the stomach of a hen, reported by Broadhurst.

TABLE 16.
Streptococcus equinus.

Source.	Holman.	Andrewes and Horder.	Floyd and Wolbach.	Hartzell and Henrici.	Broadhurst.	Total.
Throat	3	2	2	7
Pyorrhea	3	2	5
Feces (human)	4	4
Urine and urethra.	4	4
Heart valves.....	3	3
Blood.....	I	I	2
Infected foot.....	2	2
Plants (hay).....	2	2
Dog	I	I
Tonsil	I	I
Milk.....	I	I
Appendix	I	I
Fractured clavicle	I	I
Guinea-pig (peritoneum).....	3	3
Guinea-pig (blood).....	3	3
Blood, etc.	2	2
Total	26	I	2	2	II	42

TABLE 16.—*Continued.**Streptococcus equinus.*

(Variety with inulin.)

Source.	Holman.	Andrewes and Horder.	Total.
Contamination.....	2	2
Blood (endocarditis)	1	1
Eye	1	1
Ear	1	1
Sputum	1	1
Horse feces	1	1
Guinea-pig (throat).....	2	2
Guinea-pig (blood)	2	2
Total	10	1	11

Streptococcus equinus (Table 16), adopted from Andrewes and Horder's nomenclature, is represented by forty-two strains. There is no hemolysis and salicin is the only one of these four carbohydrates fermented. The sources are varied, but they often suggest air contamination. It is found in the mouth (13), in the urine (4), human feces (4), and guinea-pig invasion (6). Three hundred and sixty-five strains collected from the literature and grouped by the fermentation tests, without the test for hemolysis, probably include many that should be grouped here. Forty-six of these were grown from horse feces, fifty-three from human feces, one hundred and eighty-four from air, and thirty-nine from the mouth. Bergey's eight strains from horse manure and eight from human saliva should probably also be included. It is largely on the basis of its source being traced to horse manure that the name has been retained. Andrewes and Horder showed that this organism is highly resistant. They dried it on garnets for several months and found it still alive.

The variety with inulin contains eleven strains. Andrewes and Horder also speak of the inulin-fermenting type as a variety of the *Streptococcus equinus*. The sources of eighty-seven strains grouped by the fermentation complexes indicate that it is closely related to its group. Of these eighty-seven strains, thirty-two came from air, twenty-six from horse manure, eleven from human feces, and ten from human throat. The finding of this organism often suggests contamination from the air, and in this connection it is interesting to note that I have listed under contamination a strain grown from a supposedly-pure culture of pneumococcus, sent from another laboratory. The morphology (long chains) of this strain, the small, dry colony on blood agar and the non-fermentation of lactose, precludes its being an altered form of the original pneumococcus, which was typical in all points. It is further to be noted that two of Rosenow's strains, I and 713, reported as hemolytic streptococci, fermented lactose, and not inulin, but when "transmuted" to the non-hemolytic type failed in lactose and gained inulin. Unfortunately salicin was not used and we are unable to say definitely that these two *Streptococcus viridans* strains were *Streptococcus equinus* (variety with inulin). But the comparative rarity of non-lactose fermenters is very suggestive of contamination with air streptococci.

TABLE 17.
Streptococcus ignavus.

Source.	Holman.	Andrewes and Horder.	Floyd and Wolbach.	Hartzell and Henrici.	Broadhurst.	Total.
Throat			8		4	12
Tonsils.....	I		5			6
Throat (diphtheria)			5			5
Throat (scarlet fever).....			4			4
Abscess			4			4
Urine.....	3					3
Endocarditis			3			3
Heart's blood (scarlet fever).....			3			3
Pyorrhea				2		2
Brain and spinal fluid		2				2
Milk					2	2
Middle ear	2					2
Lung		I	I			2
Liver abscess	I					I
Tooth	I					I
Feces.....	I					I
Appendix	I					I
Joint		I				I
Blood		I				I
Pneumonia			I			I
Pleural cavity			I			I
Cellulitis			I			I

TABLE 17. — *Continued.*

Source.	Holman.	Andrewes and Horder.	Floyd and Wolbach.	Hartzell and Henrici.	Broadhurst.	Total.
Coryza			1			1
Mastoid			1			1
Scarlet fever (autopsy)			1			1
Unknown			1			1
Dog (throat)					1	1
Dog (alimentary tract)					3	3
Horse feces					1	1
Blood, etc.					3	3
Total	10	5	40	2	14	71

Variety with inulin.

Cat (esophagus)					1	1
Total					1	1

Streptococcus ignavus (Table 17) has been so named on account of its relative inactivity on blood and carbohydrates. None of the four carbohydrates used in our classification are fermented and there is no hemolysis of blood. However, I have never encountered a streptococcus which was entirely lacking in fermentative power. In my hands, they all attack either dextrose or saccharose, even if the other test substances are not acted upon. There are seventy-one strains in this group, but forty of them come from Floyd and Wolbach's tables, and the total number we believe should be

reduced. There is nothing marked concerning the habitat or distribution and it is a rather uncommon organism. I have found it only ten times.

Broadhurst reports one strain of the variety with inulin isolated from the esophagus of a cat.

Practical use and advantages of the method. — Having been deeply interested in the streptococci for many years I have felt the urgent need of a practical method for classification. The combined methods of Schottmüller and Gordon, which were, I am convinced, only partially carried out by Andrewes and Horder, offered the most promising field. The classification by the last named writers was too cumbersome, giving too many varieties to be of use in routine work. Moreover, I soon learned that for the best results a medium more favorable than plain broth must be used, and after trying modifications of Loeffler's serum with the various carbohydrates, the serum water media of Hiss, serum broth made in various ways, and other media, I decided that serum broth, made as I have previously described, is the most favorable medium for the growth of these bacteria. Litmus is not a satisfactory indicator and it was soon discarded in favor of Andrade's decolorized acid fuchsin, with the most favorable results. Titration for the determination of acid fermentation is open to too many variations, as discussed above, and is much too complicated for the routine laboratory worker. Blood agar, as originally described by Schottmüller, I found too dense a medium for careful reading of hemolytic and color changes, and I have reduced the amount of defibrinated blood from forty per cent to five per cent. It is advisable that the blood agar be made up in quantity in order that comparative work may be more carefully carried out. In going over the work of other investigators I have endeavored to eliminate the tests that were not of striking importance and have been able, without interfering with their general results, to make their findings more intelligible. The results in litmus milk are duplicated, for practical purposes, by those in lactose serum broth. Neutral red, which

has never given any particularly useful results, has been largely discarded by all. Saccharose is attacked by almost all the strains and the failure of its fermentation has not been shown to have any significance. MacConkey quotes Merck as saying that raffinose may always contain traces of fructose and other sugars. The fermentation of inulin and raffinose, as Broadhurst has shown, runs almost parallel. Inulin was omitted by her in her scheme of possible relationships of the groups, but in my opinion it is better to retain inulin, on account of its being attacked by practically all pneumococci. Coniferin has not been used by many workers, but salicin has been broadly adopted to represent these glucosides. The growth on gelatin is not satisfactory and strains of streptococci which develop poorly on isolation are able, after a few transfers, to grow well in this medium. Gelatin should be used for the liquefaction test where there is any suspicion of the *M. zymogenes* being present. Gelatin, however, is too rarely liquefied to adopt it for routine use. I have collected from the literature the fermentation complexes of 5,473 strains of streptococci. These include the strains listed in my tables. A fuller analysis of the others will appear in another paper. These strains have all been tested on the four carbohydrates I have selected and, together with several hundred streptococci which were grouped by the fermentation test on certain of these, have been largely used as a basis for my selection.

Lactose is shown to have been fermented by 5,505 strains as against 1,110 without fermentation or, in other words, there are nearly five times as many lactose fermenters as non-fermenters. Owing to the frequency with which lactose is attacked several investigators have discarded its use. Houston in his study of water streptococci only included the lactose fermenters, while Lyall tested out lactose on only 100 of his 263 strains, considering lactose fermentation positive in the others. It is, nevertheless, an important carbohydrate to retain, on account of the many strains which do not ferment it. The non-lactose fermenters were early shown by Gordon to be the commonest streptococci in the air of cities,

and the source of these air forms has been shown by several workers to be from horse manure.

Mannit is less often fermented than lactose. From the collected results it is found that 5,022 strains are recorded as failing to ferment mannit, and 1,239 as mannit fermenters. It is striking that of the mannit-fermenting streptococci, over half are from the feces or urine of man and animals, and many more are from sources strongly suggesting that they were originally from the intestinal tract. Ruediger laid particular stress on mannit by dividing his strains into two groups, according to whether they did or did not ferment it.

Salicin is frequently attacked by streptococci: 3,976 strains are reported as fermenters, and 1,729 as failing to ferment this glucoside. Andrewes and Horder believed that the fermentation of salicin had some relation to pus formation, and there is evidence that this may be true. Salicin is fermented by streptococci which do not attack lactose. These are particularly associated with the horse, in health and disease. Savage distinguished streptococci from bovine mastitis by their failure to ferment salicin, while in healthy milk he found the salicin-fermenting forms predominated. The great majority of streptococci from human saliva fail to ferment salicin.

Inulin is not often fermented by streptococci. In the collected records 5,285 do not ferment, and 743 ferment inulin. Some of these fermenters are undoubtedly pneumococci. In our classification the inulin-fermenting strains are placed as varieties of the particular group into which they would otherwise fall. The majority of streptococci do not ferment inulin, and almost all pneumococci do. There are a fair number of exceptions on both sides. The inulin test for streptococci is important, largely as a negative one.

I have, therefore, limited the tests to the disaccharide lactose, the polyatomic alcohol mannit, the glucoside, salicin, and the polysaccharide inulin. These are used in one per cent quantities in serum broth, with the Andrade indicator, and along with the five per cent blood agar, give us the differential test media. An example from Andrewes and

Horder will serve to illustrate why this simpler classification appears more logical. From the pericardial cavity of a fatal case of rheumatism these authors isolated nine streptococci of seven varieties. These streptococci were all long-chained. Two strains listed by them as *Streptococcus pyogenes* were positive in lactose and saccharose, and six as *Streptococcus anginosus*, although giving variable reactions on carbohydrates, milk, and neutral red. (One variety of the latter was positive on lactose, saccharose, neutral red, and milk, another gave the same reactions, but neutral red was negative, a third was positive on lactose, salicin, saccharose, milk, and coniferin, the fourth on lactose, saccharose, and raffinose, the fifth was the same plus coniferin, while the sixth fermented salicin, saccharose, raffinose, and coniferin). My classification and interpretation of these results would be six strains of *Streptococcus anginosus*, or more probably *S. salivarius*, two of *Streptococcus pyogenes* or *S. mitis*, very possibly a secondary invader, and one strain probably *Streptococcus equinus* as a contamination.

It is not necessary to make the study of the streptococci a special research problem, since the various strains encountered in the routine bacteriological examinations can be readily classified when isolated by the method I have outlined. It is highly desirable that more information be obtained of the numerous streptococci coming to our laboratories, and that, in experimental work, the strains used be more satisfactorily classified, so that others may be able to duplicate, if desirable, the experiments. This, I believe, can be largely accomplished by this method of classification.

Many useful conclusions may be drawn from such a classification. It may be pointed out that it is of considerable practical importance to be able to distinguish between *Streptococcus fecalis* and *Streptococcus pyogenes* in peritoneal fluids. In pus from the middle ear it is likewise important to know whether we are dealing with a severe infection by *Streptococcus pyogenes*, or a

relatively milder invasion by the *Streptococcus mitis* or *S. salivarius*. In positive cultures from the blood with a *Streptococcus viridans* infection, it adds very much to our appreciation of the case to know whether the organism is *Streptococcus salivarius*, with a possible portal of entry in the tonsils or buccal cavity, or whether it is *Streptococcus fecalis*, with the probable source the intestinal tract. The finding of *Streptococcus mitis* leaves the question of source undecided, the mouth cavity, however, being the probable origin.

There is one important point which has developed in this study, and that is that the streptococci are not specific in their disease production, at least as far as I have been able to determine. There is no evidence to support the view that only one type of streptococcus produces endocarditis or nephritis, or gives rise to septicemia in the puerperium, scarlet fever, or other conditions of lowered resistance. Neither do I believe that one streptococcus is responsible for all the ill effects in all of these cases. Streptococci of several kinds live in symbiosis in the mouth and the intestinal tract. They are very often found in mixtures in infected areas, as in the peritoneal, pleural, and other cavities. The blood stream may also be invaded by more than one type of streptococcus at the same time, although, as a rule, we only recover one type. Andrewes and Horder believed that the blood is being continually invaded by these organisms, but that they are being continually destroyed. This view has been supported by many writers, and I am convinced of its truth. It is therefore not surprising to find different organisms locating in various damaged areas. Under these conditions, by the use of the hemolytic test alone, we are liable to draw erroneous conclusions. Thus by this single method, the various members of the viridans or hemolytic groups cannot be distinguished.

There are so many interesting points for discussion that I must limit myself to a few which I believe to be important.

The streptococcic flora of the mouth has always been confusing. The finding of mouth streptococci in the air of rooms, as shown by Gordon, may be taken as indicating pollution of the air from the oral cavity, and thus the flora of the mouth is continually being replenished from the mouths of others. Another source of origin for the salivary streptococci appear to be from cow's milk, which in turn is subject to contamination from cow's feces, while still a third source is the air contamination by streptococci from horse manure. In the mouth cavity many of these strains find favorable conditions for further development. Broadhurst has shown that many streptococci being swallowed in the sputum can pass through the stomach without being destroyed in the limited time by the gastric juices. In the intestinal tract the cultural conditions are markedly different from that of the mouth and many of these strains are destroyed, while others, especially the more vigorous forms, such as the *Streptococcus fecalis* flourish and produce a flora quite different from that found in the mouth.

It cannot be too often repeated, however, that the entire flora of any region of the body may be suddenly changed by alterations in the food supplied to the bacteria, the reaction of the secretions, the presence of inflammation, and many other important environmental changes. Particular streptococci, finding these new conditions favorable, multiply rapidly, and the former inhabitants are crowded out. In diphtheria, for example, streptococci of different types may grow rapidly and may seriously complicate the conditions, as has been repeatedly pointed out by Le Gros and others. Although the streptococci present in the greatest numbers in normal saliva and intestines are of the viridans group, a careful search will almost always reveal members of the *Streptococcus hemolyticus* group and these, as experience teaches, are the strains most capable of producing severe infections. In this connection the influx of strains from disease sources outside of the body where the streptococci have developed to a high degree their pathogenic characters must not be forgotten. It is beside the point to argue that streptococcus infection in

rheumatism and other diseases is a secondary invasion. It is most certainly true that in practically all of our infections the bacterial attack is secondary to the necessary conditions of lowered resistance. In many of these arguments one is reminded of Pettenkofer's demonstration to prove that the *V. cholera* does not always cause epidemics of cholera. This author, in Munich, drank one cubic centimeter of a twenty-four hour broth culture of a strain of a comma bacillus isolated from an epidemic in Hamburg. The result was an attack of diarrhea, but no other symptoms of cholera. Emmerich carried out the same experiment and had a more severe attack. In both cases pure cultures of the cholera vibrio were isolated from the stools. Pettenkofer used this experimental proof to support his theory on the occurrence of epidemics. He believed there were three requisites, (x) the presence of the germ, (y) local conditions of time and place, and (z) the individual disposition. His experiments were undertaken to show the importance of the last two requirements.

The conditions of lowered resistance in the *Streptococcus viridans* infections are receiving much attention, but we are only on the threshold of this study. When the streptococci have established themselves within the system, we are usually at a relatively late stage of the disease. Libman has shown that in infectious viridans endocarditis the cases may become spontaneously bacteria free. The work of many of the German investigators would indicate, though on somewhat dubious grounds, that the viridans endocarditis is almost always eventually fatal. There is no doubt that with careful blood cultures the frequency of viridans infection in the earliest stages will be definitely shown and the recurrent attacks guarded against. It is most important that we should know the streptococci causing these infections and that they be classified by the above method as offering something tangible with which to work.

It may be necessary to further subdivide these groups. If there be any hope in the use of immune tests for further differentiation some such classification must be followed and

the same is true for the application of vaccine and other specific treatment.

Constancy of the tests. — In the practical application of this method of classification, the question of constancy must be considered. There are certain important points bearing directly on this problem, which should be emphasized.

1. Pure cultures: There is strong evidence that in many of the examples, which have been used as indicative of so-called alterations, mixtures of different streptococci were present in the original cultures. Gordon, Floyd and Wolbach, and others, have also pointed out this source of error in constancy tests. I have, in a former article, shown some of the curious results which may be obtained when working with such mixtures. The organisms with the dominant characters are usually so striking as to be easily recognized. At other times combinations of reactions are found. However, a streptococcus with the power to ferment a certain carbohydrate may be overgrown by another form lacking this fermentative ability, although this finding is uncommon. After the culture is kept for some time, one or other of the strains will die out and our subsequent tests show a set of reactions which are quite different.

2. Vigor of growth: The less favorable culture media, such as plain broth used as a base, will retard the manifestations of the fermentative ability of many strains. However, after repeated transfers the streptococcus may become accustomed to this environment and retests will show a more vigorous growth and often a wider range of substances attacked. Similar results are met with on blood agar where the basic agar is unfavorable or the blood is too old.

3. Time of growth: The reading of the reactions at an arbitrary and insufficient time limit is a common source of error leading to confusion, similar to the above. A

streptococcus, which may not ferment mannit until the fifth day or later, will, after prolonged cultivation, ferment it in a few hours. This has undoubtedly led to many erroneous conclusions. A streptococcus must be thoroughly tested for its fermentative ability beyond the time limit adopted for the particular experiment. Otherwise, the appearance of an alleged new quality cannot be accepted as such, since it admits of interpretation as being merely a more vigorous and more rapid growth. Broadhurst, who has contributed the most complete work on the question of constancy of streptococci, has overlooked this point and most of her variable results may be thus interpreted. She has shown, for example, that several of her strains in her three-day tests in meat broth gave certain fermentation complexes. New fermentation characters appeared to develop after submitting these strains to the stimulating environment of growth in saliva or the alimentary canal. The same changes occurred, however, in her control stock culture after transfers over a long period, and the strains showed a general increase in luxuriance of growth, as indicated by the cultures in gelatin. I am not convinced that many of these changes are anything more than the stimulation of characters which would have naturally shown themselves in more favorable media, or after longer cultivation. The subjection of pure cultures to the conditions in the saliva and intestinal tract will, as she has shown, cause either the death of the strains or stimulate a more vigorous growth. The gain of mannit fermentation, as shown in her chart in a series of three-day titration records, and the greater constancy of the saccharose-lactose-salicin-mannit complex, are not surprising when we consider that the streptococci she was studying were largely from animal sources where *Streptococcus fecalis* is common. In carrying out such experiments the fermentation tests should be done under all possible cultural conditions. The fermentative abilities of the strain must primarily be definitely established, by growth in favorable media, as serum broth, aërobically and anaërobically, and over long periods of time, before we can consider the possibility of mutation or alteration. It is

clear from her work and that of others, that the majority of strains grow well in the simpler media, and the results may be taken to indicate the average fermentating characters of the streptococci. It is for this reason that I have included these strains in my tables to indicate the general trend of fermentation complexes.

4. Animal passage: The presence of many varieties of streptococci in the alimentary tract of guinea-pigs, rabbits, dogs, cats, horses, cows, and practically all animals, and the high natural invasive power of these organisms, make animal passage of streptococci practically impossible to control. I do not wish to be misinterpreted as saying that animal passage of many bacteria is not a useful method of study. However, if the experimental animals are shown to harbor bacteria, similar, or closely related to those used in the experiment, or are particularly liable to spontaneous infection by these bacteria, then the results, through the use of such animals, are open to serious criticism unless the organism used in the experiment can unquestionably be recognized when recovered.

CONCLUSIONS.

1. A simplified and practical method to classify streptococci is desirable and the combination of Gordon's carbohydrate fermentation and Schottmüller's blood agar tests, modified for practical purposes, offers the most useful means to this end.

2. Constancy of these reactions is essential to the method here advocated. Evidence of transmutation or examples of inherent alteration of character have been insufficient to invalidate this method.

The confusion in the results of these tests, and most of the examples of so-called alterations, are explainable on the relative difficulty of growth and the morphological similarity among the different types. Mixed cultures are difficult to detect and often hard to separate, and the strains vary widely in their longevity and resisting power. Alterations

in vigor of growth must be guarded by using the most favorable media and extending the time of observation. Animal experiments are unreliable, owing to the high invasive power of streptococci of the animal itself.

3. Standard methods should be followed. The carbohydrate serum broth, described by the author, and five per cent defibrinated human blood agar, offer the best media for this purpose. Quantitative carbohydrate acid tests by titration are not as useful as the qualitative tests. They add considerable unnecessary labor, without any corresponding advantage. Andrade's decolorized acid fuchsin is an eminently satisfactory indicator for qualitative tests.

4. The classifying of streptococci by the method here outlined can be carried out in the routine bacteriology of any laboratory. It is unnecessary to make it a special research problem.

5. Our method for carrying out the classification is briefly as follows: All material is cultured in serum broth before plating on blood agar. The cultures are tested on blood agar slants for hemolysis, and in lactose, mannit, salicin, and inulin serum broth for fermentative power, over a period of at least seven days.

6. By this method of classification we recognize the hemolytic and non-hemolytic group, under each of which eight sub-groups are arranged (see Chart 1).

7. Much information of practical importance concerning streptococci is made available by the use of this method of classification. Many of the air streptococci can be traced to their sources and the same is true of streptococci found in milk, the mouth, the intestinal tract, animal tissues, and other places.

8. The individual groups of streptococci are not specific in their disease production. The members of the hemolytic group are commonly more virulent and pathogenic, producing more rapidly progressive disease processes than those of the viridans group.

9. Almost all streptococci have relatively high invasive powers, and the varying conditions of lowered resistance

play a most important rôle in determining the type of infection. This is especially true in the chronic infections.

10. Focal areas of streptococcus infection often contain more than one type of streptococcus. The apparent alteration of character of the streptococci, in these cases, is due to the confusion arising from the mixtures. In the mouth, intestinal tract, the vagina, and in other regions, the entire flora, including the streptococci, may rapidly change with the alteration of the local environment.

11. It is believed that with the adoption of this classification greater uniformity will be established for the comparative analysis in the study of streptococci.

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SPONTANEOUS INFECTION IN THE GUINEA-PIG

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SPONTANEOUS INFECTION IN THE GUINEA-PIG.*

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The guinea-pig is probably more commonly used in laboratory studies than any other animal. It is important, therefore, to know very thoroughly the diseases to which it is liable, the bacteria commonly found in the naturally infected regions of the animal, those which may and do invade the animal tissues, the liability to infection with extraneous organisms, and the post-mortem findings in spontaneously dead or untreated animals. There have been numerous reports in the literature on epizootics among these animals with isolated references to organisms grown from guinea-pigs in health and disease, and particular emphasis has been given to certain organisms as the specific cause of the infectious conditions, but there is no general discussion on the bacteria associated with guinea-pigs.

In all animal experiments the ever-present liability to spontaneous infection must be carefully considered, not only in regard to the infection that may be present before an inoculation, but the stimulating into activity of latent infection, or the lowering of the resistance by the treatment and the invasion of the tissues by bacteria from the animal itself. There are many studies reported in which these

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considerations have been overlooked, and results have been obtained which would appear disturbing were they not explainable along these lines.

It is with the object of bringing together in as concise a form as possible the more important facts on the bacteriology of guinea-pigs, the diseases to which they are liable, and the chances of wrong conclusions after experimental work, if these facts are not appreciated, that this paper has been prepared. The material studied was obtained from a breeder of guinea-pigs who has a stock varying from about five hundred to seven hundred. The animals in his pens are exceptionally vigorous and healthy. They are given the greatest care in respect to ventilation, heating, and food. In the breeding pens one male is kept with a dozen females, which will partly account for the greater proportion of females in these studies. The pens are built of wood. The water basins are outside the cages with an opening small enough to prevent the animals getting into the water or spilling the basin on the floor. The consequence of this arrangement is that the cages are kept reasonably dry, which is most important in preventing the spread of infection. The bin for the mixture of bran, coarse corn meal, and middlings feeds automatically and prevents the food from being spread over the floor. The rack for hay is also outside of the cage. The cages are cleaned regularly, lime is powdered on the floor, and a bedding of straw provided. A shelter box, for the protection of the young, adds considerably to the comfort of the animals. During the summer a liberal supply of green food is provided and in the winter months sprouted oats are continually supplied with variations of cabbage, carrot tops, and other available green food from the market. The heating of the house is by means of gas stoves and the ventilation by an overhead flue with curtains and other devices to regulate it. Nevertheless, although so many of the living conditions are excellent, the danger of an epizootic in such wooden cages in tiers is very great. Fortunately no true epizootic has occurred and the

number of deaths or cases of sickness has not been above what one would expect among such a stock of animals.

I have had the opportunity in the last two years of studying about two hundred guinea-pigs from this source. The animals were brought to the laboratory shortly after death, or when evidently ill. The latter were watched until death ensued, or more often were chloroformed. Autopsies were made on all these animals and cultures taken from the various cavities and tissues. In the majority of autopsies pieces of tissue from the organs were kept for histological study, which will serve as the subject for a future contribution. In the present paper only the briefest references will be made to the gross pathological findings. The cultures from these animals were grown, as a rule, in serum broth with or without carbohydrates, those from the trachea being also made on plain agar slants. In practically all of the cases smear blood-agar plates were made from the fluid media, plain agar being used only when no evidence of streptococcus or pneumococcus infection was present.

My interest at the beginning was particularly attracted to the streptococcus-pneumococcus group of bacteria, but all organisms encountered were carefully studied. By the use of the above technic I have clearly demonstrated the frequency of primary and secondary infections by the streptococci in these animals. There is no doubt that without the use of serum broth and blood agar very many of these infections would have been overlooked. Over five hundred and seventy-five pure cultures of various organisms have been isolated from these guinea-pigs and carefully studied.

Since the main object of this report is the bacteriology of guinea-pigs, I will group my observations under the more important bacteria which have been reported in the literature or found in this series, but first I will briefly discuss the normal findings in apparently healthy animals.

The mouth cavity of guinea-pigs shows a great variety of

bacteria, both cocci and bacilli. The bacilli are of various sizes and lengths, many of them resembling the so-called *B. maximus* of the human mouth. Dark field illumination reveals spirochetes somewhat like the *Spirocheta dentium*, but usually somewhat longer.

Binaghi saw micrococci and large motile bacilli, and cultivated a *Staphylococcus albus* from the sputa of many normal guinea-pigs. Kaspar and Kern always found cocci, Schiller, *Staphylococcus aureus* and *citreus*, M'Gowan, Ferry, and Smith, *B. bronchisepticus*. I have isolated from the mouths of guinea-pigs several types of streptococci, principally *Streptococcus fecalis* and *Streptococcus equinus*, with their inulin varieties. In the lower respiratory tract bacteria are, of course, much more uncommon. Beco found the tract sterile from the middle of the trachea down. Boni found the lungs were usually sterile, but in many cases a few organisms, some pathogenic, such as the pneumococcus, are present. Selter found in the lungs pneumococcus, *B. cavisepticus mobilis* (*B. bronchisepticus*), the hay bacillus, and other spore bearers. Ferry found *B. bronchisepticus* in the lungs. Arlo isolated from the lungs or bronchial glands staphylococci, *albus*, and *citreus*, *B. coli*, and cocci resembling *M. crassus*.

I have isolated *B. bronchisepticus* from the trachea of apparently healthy animals. Graham-Smith found *B. xerosis canis* in the eyes of healthy guinea-pigs.

The intestinal tract is naturally very rich in microörganisms.

Bacilli of the paratyphoid group have been isolated from normal feces (Marshall and Morgan) and from "carrier cases" (O'Brien, Uhlenhuth, and Hübener). *B. coli* is reported from guinea-pig intestines by Harris and Eyre, *B. acidophilus* by Mereschkowsky, a capsulated bacillus by Jensen, *Staphylococcus albus* by Schiller, streptococcus by Bergey, ameba by Walker and Nägler, schizophytes by Chatton and Pérard, cercomonas by Perroncito, trichomonas by Galli-Valerio, and pseudospirochetes (free undulating membranes of trichomonaden) by Nägler, and Kline (1891) said that comma bacilli are found on the mucous membranes of healthy guinea-pigs. The penis, anus, mammæ, and vulva were shown by Cowie to yield smegma bacilli.

In the vagina Wagner found streptococci. From the intestinal contents I have isolated varieties of *B. coli* and several streptococci (*Streptococcus fecalis*, *Streptococcus fecalis* with inulin, *Streptococcus non-hemolyticus* ii.). Having thus briefly reviewed the bacterial flora of different

parts of the guinea-pig I will consider the occurrence of the main groups of bacteria in these animals.

The coccaceæ as they occur in guinea-pigs include staphylococci, *Micrococcus tetragenous*, streptococci, and pneumococci.

STAPHYLOCOCCI. — The staphylococci are apparently uncommon in guinea-pig infections, being chiefly noted in mixed cultures (Perkins, Wagner, Barnabo) or in the naturally infected parts of the animal.

The three common varieties of staphylococci have all been found in the buccal cavity, the albus by Binaghi and Arlo, the aureus by Schiller, and the citreus by Arlo and Schiller. The latter author found the *Staphylococcus albus* in guinea-pig feces and Ford reported staphylococci from the liver.

In my studies I have isolated *Staphylococcus albus* from seventeen guinea-pigs, four times from the peritoneal cavity, four times from the heart's blood, three times from the trachea, twice from the pleural cavity, and once each from the lung, liver, uterus, and a subcutaneous abscess. They are usually associated with other bacteria and appear to have little significance. *M. zymogenes* was found with other bacteria in the eye of one animal.

MICROCOCCUS TETRAGENOUS.

This organism has been reported as the cause of a contagious disease in guinea-pigs by Altana. He had only four cases and could not reproduce the disease with the organism isolated. The animals retained their appetite, but were greatly emaciated at death. Kaspar and Kern also describe an epizootic caused by this organism. The disease lessened in severity as the epizootic progressed. In the beginning it was very acute, but later became of a chronic type with numerous abscesses in different parts of the body. This observation of an epizootic with acute death in the beginning, and later with chronic types of disease, has been noted by several authors. Forty-seven animals died and *M. tetragenous* was found in pure culture in all but three. In two it was mixed with a Gram negative bacillus and in one with pneumococcus. Males and females were almost equally affected.

I have isolated *M. tetragenous* from the peritoneal cavity of but one animal.

STREPTOCOCCI.*

Probably the first report of streptococcus infection among guinea-pigs is that of Eberth in 1885. In his study of the disease pseudotuberculosis of guinea-pigs, he described small areas in the liver, spleen, and abdominal lymph glands. There was round-celled infiltration about these areas and micrococci, usually in short, but occasionally in long chains, were seen. He was unable to grow the organism, but from his illustration he undoubtedly had streptococci. Chantemesse (1887) and Dor may have had the same organism, as it is described as being oval, and occurred singly or in short chains. These reports on pseudotuberculosis are usually grouped under infections by the paratyphoid group of bacilli.

There have been several epizootics reported of streptococcus infection among guinea-pigs, and curiously enough the diseases found were quite different in the various reports. Weber found the lungs chiefly affected, Wagner, the uterus, Boxmeyer and Flexner, the lymph glands, Horne, various organs, and Teacher and Burton considered it the cause of infectious abortion. The report by Weber included two outbreaks. The first began in June and July among the rabbits and later attacked the guinea-pigs, and the second occurred in the following March with thirty-one deaths. Autopsies on thirty animals showed red hepatization of the lungs to be the most common occurrence. Pregnant females were chiefly attacked and the streptococcus was isolated from the lungs, the respiratory tract, and the heart's blood. The epizootic of puerperal disease reported by Wagner is based on the study of twenty-one cases. In fifteen the uterus was the chief organ involved, while in four the lungs were also attacked, and in one of these a large liver abscess was found. Two pigs were negative. The cultures gave a pure growth of streptococci in eleven, mixtures in four, *B. proteus* in two, and no growth in three. The mixtures were streptococci with proteus-like bacilli twice, and with staphylococcus twice. Two of the streptococci did not hemolyze when the cultures from hydrocele broth were added to a five per cent emulsion of rabbit's red cells. The injection of these streptococci into the uterus reproduced the disease, while strains isolated from the vagina of pregnant and non-pregnant animals failed to do so. The disease was most prevalent from October to December and from March to April. These two forms of the infection are of particular interest and are quite similar to those which I have had, and which were caused by hemolytic streptococci.

The extensive epizootic of lymphadenitis (lumps) reported by Boxmeyer is important as it indicates a quite different type of disease and one which I have not encountered. The disease attacked many hundreds of animals and was characterized by marked swelling of the lymph glands, often with abscess formation. From the results of over one hundred autopsies it was found that the cervical glands were involved in over ninety

* The nomenclature used for the members of the Streptococcus group is based on my recent paper. Jour. Med. Res., 1916, xxxiv, 377.

per cent and the axillary and inguinal glands less frequently. Abscesses occurred fairly often in these glands as well as in the spleen, liver, uterus, and lungs. The disease was considered as a wound infection and streptococci were found in about two hundred animals, mostly in pure culture. These streptococci closely resembled those found by me in severer infections and which belong to the *Streptococcus pyogenes*. Boxmeyer reported that his strains were hemolytic, grew in long chains, fermented dextrose, maltose, and lactose, and did not ferment mannit. The sixteen strains studied by Lamar and which were isolated from an epizootic similar to Boxmeyer's were also hemolytic. Two epidemics reported by Horne, occurring in 1896, and again in 1903, were apparently contracted by the guinea-pigs from lemmings which had been brought into the laboratory during the curious migrations of these animals. A streptococcus was found in both the lemmings which died and the guinea-pigs, fifty of which died in the former outbreak, and seventeen in the latter. The autopsy findings were chiefly enlargement of the spleen, adrenals, and lymphatic glands, and hyperemia of the lungs with hemorrhagic and at times necrotic pneumonia. The streptococci seen by Teacher and Burton in sections of the placenta and uterus were not grown in pure culture. Theobald Smith mentions having grown a streptococcus in pure culture from a guinea-pig in 1903, the organisms having been seen in sections of the highly congested lungs and the glomeruli of the kidneys. Bergey included two strains of streptococci from guinea-pig feces in his study of fermentation characters of this group.

In my own work, streptococci of various types have been isolated from ninety-four guinea-pigs. The *Streptococcus pyogenes* from the heart's blood in twenty-seven, from the pleural fluid in forty, from the peritoneal fluid in sixteen, from the uterus in ten, from the trachea in three, from the eye in two, from the lung in two, and from the pericardial cavity, vagina, peritoneal abscess, cervical abscess, mammary abscess, thyroid abscess, liver, and urine in single cases. The principal disease from which the *Streptococcus pyogenes* was isolated was pneumonia, forty-nine animals having had the lung affected. The extent of the involvement in the lungs varied greatly. In the more typical cases whole lobes appeared in the stage of red hepatization, the pleural cavity was filled with bloody fluid resembling hemolyzed blood, and the nose and mouth were stained red from an expulsion of blood which frequently occurs just before the death of the animals. However, all grades of pneumonia

and pleurisy have been encountered, from an acute congestion to empyema with half the pleural cavity walled off and filled with dirty pus. One interesting case was that of a breeding female which was believed to have had two former attacks of pneumonia. She supposedly had had one previous attack in September, 1914, while pregnant, gave birth to her young, but never recovered her weight. Death took place February, 1915, and the autopsy revealed the entire right lung in the stage of red hepatization with small necrotic areas over the surface. There were also numerous firm fibrous adhesions between the lung and the parietal pleura. *Streptococcus pyogenes* and *B. lactis aerogenes* were isolated from the pleural fluid and heart's blood. No growth was obtained from the peritoneal cavity.

The next most common condition was that of acute endometritis, which was found in ten animals. *Streptococcus pyogenes* was isolated from the uterine cavity and the heart's blood in the majority. These cases closely resembled the cases of human puerperal infections, as well as those reported by Wagner in guinea-pigs. In one case the uterus was intensely inflamed and contained a large quantity of pus, the purulent discharges also filling the vagina. Cultures from the uterine cavity gave a pure growth of the *Streptococcus pyogenes*, while those from the vagina showed besides the *Streptococcus pyogenes* also the *Streptococcus fecalis* and *Streptococcus mitis*. These findings are similar to many to be found in the literature from human cases which have been the basis for much controversy over the relationship of hemolytic and non-hemolytic streptococci to puerperal infections. No growth was obtained from the heart's blood in the above case. Another uterus infection in which *Streptococcus pyogenes* and *B. lactis aerogenes* were grown from the uterus showed the latter organism with *Streptococcus fecalis* in the heart's blood. Several small abscesses in the uterine ligaments, with ulcers in the uterus, and an acute inflammation of the mammæ in another pig, gave pure cultures of the *Streptococcus pyogenes*. One of the most

interesting cases in this series of uterine infections by *Streptococcus pyogenes* was that in which a marked thrombophlebitis of the uterine vein occurred. The uterus showed a purulent endometritis and the uterine vein on the right side showed complete thrombosis as far as the vena cava. The thrombosed vein appeared as a firm, yellowish cord and there was extensive periphlebitis. The thrombus did not extend into the vena cava. In this case there was also a very small ulcer in the appendix. Cultures from the peritoneal cavity, liver, heart's blood, and uterus yielded pure cultures of *Streptococcus pyogenes*.

Five different abscesses were found with *Streptococcus pyogenes*, either pure or mixed with other organisms. One in the neck was large and fluctuating and contained a milky, foul-smelling fluid with numerous bacteria. *Streptococcus pyogenes* was isolated from the mixture. The animal recovered after the abscess was opened and drained. In a second, an abscess in the liver with thick fibrous walls, bound to the peritoneal wall by firm fibrous adhesions and containing thick inspissated pus, occurred in an animal dying of a typical advanced pneumonia. *Streptococcus pyogenes* was grown from the pleural fluid and heart's blood. The third case was a large female brought to the laboratory with a swelling in the left mamma. This, when opened, contained a dirty necrotic material, much foul-smelling gas, but no true pus. Cultures gave *Streptococcus pyogenes*, *Streptococcus fecalis*, and *B. lactis aerogenes*. The following morning the animal was very ill and was chloroformed. An autopsy revealed the necrotic area in the mamma well walled off, a greatly enlarged dark red and soft spleen, a fatty liver, and nothing else of particular importance. Cultures from the heart's blood gave a pure growth of *Streptococcus pyogenes*. A fourth animal showed at autopsy, besides a typical pneumonia, a small abscess in the pectoral muscle, and a mass of nodules of various sizes, containing inspissated pus in the wall of the left horn of the uterus and in the parametrium; some of the latter were bound fairly firmly to the wall of the colon. Cultures from the uterus and pleural fluid gave pure

growths of *Streptococcus pyogenes*, while those from the peritoneal fluid yielded *Streptococcus fecalis* and *B. acidilactici*. Direct smear from the nodules in the uterus showed pus cells, débris, and Gram positive cocci in groups and short chains, but no acid-fast bacteria. The fifth animal had an abscess in the thyroid gland which gave a pure culture of *Streptococcus pyogenes*. This young female pig had an extremely hard liver, a congested kidney, some small, deeply congested areas in the lungs, greatly enlarged cervical glands, and intensely inflamed thyroids containing pus.

A small white pig which showed at autopsy a hard, firm liver, hypertrophied mesenteric glands, hemorrhagic kidney, and red infarct-like areas in the lungs, gave a pure culture of *Streptococcus pyogenes* from the heart's blood. In two cases with severe conjunctivitis, *Streptococcus pyogenes* and other bacteria were isolated from the eye.

A number of experiments with a strain of this streptococcus, isolated on January 9, 1915, from the pleural fluid of a typical case of pneumonia, showed it to be highly pathogenic to guinea-pigs. The animals used in these experiments were obtained from an entirely different source. On January 22 the growth from one twenty-four hour dextrose serum broth culture injected intraperitoneally killed a medium-sized female pig in less than eighteen hours. A similar result followed the same intraperitoneal dose in a guinea-pig which was immediately injected with a mixture of cholesterin, sodium oleate, and human serum. Smears from the peritoneal cavity showed in both cases large numbers of streptococci, but no cells. The heart's blood was sterile in both these animals, the organism being recovered from the peritoneal fluid. On January 29 an intraperitoneal injection of half the growth from a twenty-four hour dextrose serum broth culture killed a medium-sized female guinea-pig in about fifteen hours. An acute peritonitis was found and the organism was recovered from the peritoneal fluid and the heart's blood. On January 29 one-eighth of the growth from a twenty-four hour dextrose broth culture of the original streptococci injected intraperitoneally killed a medium-sized

female pig in about sixty-three hours. An autopsy revealed a purulent peritonitis and the organism was recovered from the peritoneal pus and the heart's blood. *Streptococcus fecalis* and *B. coli communior* were also isolated from the peritoneal culture. On February 2 a similar dose, together with the cholesterin oleate serum mixture, killed an animal in about seventeen hours. The culture from the heart's blood gave a pure growth of the streptococcus, while the peritoneal fluid remained sterile. The invasion of the peritoneal cavity in the former case by organisms from the intestinal tract is of interest.

The infection with *Streptococcus pyogenes* was found fairly evenly distributed throughout the months of the year. Improvement in ventilation, however, reduced the number of cases by almost one-half, and the acute infection of the lungs became very rare in the second year of this study. *Streptococcus equi* was isolated from a cervical gland of a guinea-pig over seven years ago.

The next most frequent type of streptococcus was the *Streptococcus fecalis* and its variety with inulin. The former was grown from twenty-six animals, from the peritoneal fluid nine times, from the heart's blood seven, from the trachea six, from the uterus twice, and once each from the pleural fluid, abscess of mamma and vagina. The variety was found in eight animals, four from the peritoneal fluid, two from pleural fluid, and one each from the heart's blood, uterus, and trachea. Both these streptococci have also been isolated from the intestinal contents and the mouth cavity of guinea-pigs. The *Streptococcus fecalis* was isolated from mixtures in all but seven cases. In one case it was grown from the peritoneal fluid after an intraperitoneal injection of *B. coli*, and in another from the heart's blood following injection with *Streptococcus non-hemolyticus* ii, showing the ease with which spontaneous infection may occur in experimental work. In two very young pigs apparently normal at autopsy it was grown in pure culture from the heart's blood. In two animals with pneumonia, which gave *Streptococcus pyogenes* from the heart's blood and the pleural fluid, the *Streptococcus*

fecalis mixed with Colon bacilli was isolated from the peritoneum. Twice it has been isolated from mixtures with *B. lactis aerogenes* from the blood, four times from the peritoneum, and once from pleural fluid and trachea. The variety has been found twice with *Streptococcus fecalis*, once pure in pleural fluid and once in the uterus, twice mixed with *B. lactis aerogenes* in peritoneal fluid, and once with *Streptococcus pyogenes* and *B. coli*, following an injection with the former organism. *Streptococcus mitis* was encountered in seven animals. It was obtained in pure culture, once from the heart's blood after injecting the peritoneal fluid of a guinea-pig previously injected with a mixture of pneumococcus and *Streptococcus fecalis*, but which had failed to show any growth from the peritoneal fluid. It was also found in pure culture from the pleural fluid of an animal in which it was also present with *B. coli* in the peritoneal fluid and heart's blood. A guinea-pig injected with a pure culture of *B. coli* gave in cultures from the heart's blood the *B. coli*, *Streptococcus equinus* and *Streptococcus mitis*. After a subcutaneous injection of *Staphylococcus pyogenes aureus* there was isolated from the resulting abscess the staphylococcus as well as *Streptococcus mitis* and *Streptococcus infrequens*.

The *Streptococcus equinus* has only been found in three animals. Once it was isolated from a mixture with *B. coli* from the heart's blood of a pig dying as a result of an injection of a pure culture with *B. coli*. In another case it was obtained from the peritoneal fluid mixed with *B. coli*. The third culture was from the liver of a small female guinea-pig and was mixed with *Staphylococcus albus*. This animal showed subcutaneous petechial hemorrhages, general glandular enlargement, a very firm liver, a thrombophlebitis of the common iliac vein with the thrombi extending into the right and left iliacs, and firm, grayish red, small areas in the lungs. *B. bronchisepticus* was grown from the trachea and no growth was obtained from the heart's blood. The variety of *Streptococcus equinus* has been isolated from the buccal cavity of a healthy pig.

This condensed report of the occurrence of the non-hemolytic streptococci in guinea-pigs will serve to show how frequently they may be encountered, and the importance of differentiating the streptococci for recognizing the contamination of cultures in animal passage. There is evidence to show that former workers neglected these points, with unfortunate interpretations. It is clearly seen from my results that spontaneous infection occurs both in untreated animals and in those injected with other bacteria. Similar results are reported for the pneumococcus as noted below.

PNEUMOCOCCUS. — The pneumococcus has been reported quite frequently, and, as Theobald Smith says, may be regarded as a widely disseminated pathogenic organism among guinea-pigs.

Binaghi (1897) reported a spontaneous infection with an organism which he called *Streptococcus capsulatus*. The animal had a peribronchial pneumonia with small abscesses in the lungs. The cocci were round and occurred in pairs and in chains of four to six cocci, the capsules were readily stained, long chains were developed in broth, dewdrop colonies on agar, and no growth was obtained on gelatin, blood serum, or potato. The organisms soon died out on artificial media.

Pneumonia is a relatively common finding in the epizootics of pneumococcus infection, as indicated in the reports of Stephansky, Tartakowsky, Wittneben, Christiansen, and Smith. The epizootic of Stephansky occurred during the winter months in Odessa, and resulted in forty deaths. The important findings from eighteen autopsies were in the thoracic cavity and included pneumonia, pleurisy, pericarditis, and abscesses in the lymphatic glands, the lungs being involved in fourteen of the animals. A few showed purulent peritonitis and one an abscess in the uterus. The pneumococcus was isolated from the lung, liver, spleen, and blood. It showed typical morphology and the capsules were stained from direct smears and from early cultures. It grew fairly well on media, coagulated milk, and did not grow on potato. Tartakowsky's outbreak, reported from Petersburg, is described as a pleuropneumonia, and the diplococci were isolated from the pneumonic lungs and the pleural exudate, and were but rarely found in the other organs. It is interesting to note that in a previous epizootic he found *B. bronchisepticus*, a finding similar to that of Theobald Smith in Boston and that of Selter, both of which are discussed below. From Bonn, Wittneben reported the loss of twenty-four guinea-pigs during the winter months. In twenty-one the lungs were involved and in thirteen the pleural cavity contained a bloody, seropurulent exudate

which is similar to that found by Christiansen and Smith with pneumococcus infection, and by myself with streptococcus. Wittneben, also, found peritonitis in eighteen and pericarditis, nephritis, and subcutaneous edema in others. The streptococcus lanceolatus was grown in pure culture from the pleural fluid, lung, heart's blood, and peritoneal fluid. Long encapsulated chains were sometimes seen. The growth on blood agar was green with a gradual clearing near the colony, and acid was produced from dextrose and lactose, capsules were easily stained and were also seen in collargol preparations.

The epizootic which Christiansen reported began in the summer, and twenty to thirty guinea-pigs died. Besides the pneumonic condition found in the lung and the hemorrhagic pleural exudate, isolated cases of peritonitis, concomitant or alone, and metritis were seen. The author had definite evidence that the infection spread from a previous epizootic among calves and a complete comparative study of the pneumococci isolated from both groups of animals showed them to be closely related. The pneumococci gave no hemolysis and fermented lactose, salicin, inulin, and raffinose, but did not ferment mannit. Theobald Smith reported pneumococcus infection among three different collections of guinea-pigs. The first case occurred in 1908, and the infection has been found every year since. During 1908 and 1909, breeding females, following parturition, died from pneumonia, with blood-stained pleural fluid. These cases were diagnosed as old pneumonia, due to *B. bronchisepticus*, with a secondary invasion of the pneumococcus. In 1913 thirty-six cases were found, and from seven of the animals only the pneumococcus was isolated, while from eleven the pneumococcus and *B. bronchisepticus* were found together. One interesting case of an abscess of the middle ear, with apparent involvement of the semicircular canals, showed capsulated cocci in the direct smear from the pus of the ear.

I have seen three guinea-pigs which showed somewhat the same clinical picture. Greatly enlarged cervical glands were found in one, and pus in the middle ear from which *B. lactis aerogenes* was isolated in two others. The diagnosis of the pneumococcus in Smith's cases was made from culture, section, and smears. The cultures were typical and the pathogenic effects of the organisms were found to be feeble.

It would appear that the pneumococcus is widely distributed and is present in apparently normal animals, as Boni found it in the lungs of healthy guinea-pigs, and Selter isolated this organism in pure culture from the lungs and liver of one guinea-pig, mixed with *B. cavisepticus mobilis* (*B. bronchisepticus*), from the lungs, liver, spleen, and kidney of another, and from the lungs and kidney of a third. These animals were considered as normal. The invasion of the pneumococcus, following inoculation or

spontaneously, is further indicated by the findings of this author. Three guinea-pigs had been inoculated with *B. dysenteriae*, killed tubercle bacilli, and *B. pseudodiphtheriae*, and died after two, four, and fourteen days respectively. A fourth animal which had not been treated died spontaneously. The pneumococcus was isolated from all these animals. These infections occurred in May and in the following winter five more guinea-pigs succumbed with a pneumococcus infection, either with or without previous treatment.

Rosenow in his article on transmutation within the streptococcus-pneumococcus group reported results which are more logically interpreted as an invasion from the animal itself, as in Selter's findings, than as transmutation of the inoculated streptococci. Spontaneous infection of guinea-pigs, as already shown, is not uncommon, and the injury resulting from an inoculation would easily lead to such invasion. Moreover, the constancy with which pneumococci were eventually found in his experiments is a strong argument for this explanation. It would appear that the pneumococcus was a much more frequent invader at the time of his experiments than were any of the streptococci, although he encountered a *Streptococcus viridans* a number of times. In this connection it may be noted that Rosenow had been carrying out extensive animal experiments with the pneumococcus during many previous years. There is one other important report on pneumococcus infection in which, however, the uterus was the principal organ attacked. This was by Richters (1913). The epizootic occurred in a breeder's stock and many animals died. There was, as a rule, a marked metritis, and in the uterine mucous membrane were numerous small tumors which contained a dark necrotic material. Among the other findings at autopsy were hemorrhages in the kidney and under the endocardium. Pure cultures of the pneumococcus were studied from these cases. There are in the literature other isolated references to pneumococcus infection among guinea-pigs. Salomon (1908) included in his tables three strains of pneumococcus from a guinea-pig epizootic. Ungermann (1911), in his experiments on natural immunity to pneumococcus, used a strain isolated from the purulent exudate of a guinea-pig which died of a spontaneous pneumococcus infection, and Kaspar and Kern (1912) stated that they had formerly observed spontaneous infection with pneumococcus in their guinea-pigs, and in one animal, in their report on *Micrococcus tetragenous* infections, they found the pneumococcus mixed with this organism.

It is surprising that in my work the pneumococcus has been found so rarely. The guinea-pigs were not subject to natural contagion from animals treated with the pneumococcus since they were far removed from any laboratory. On only three occasions have I met with organisms suggesting the pneumococcus. The first case was a small animal with

a marked pulmonary congestion. Peritoneal and pleural fluid gave no growth. The heart's blood gave *Staphylococcus albus*, and a small, Gram positive, slightly elongated diplococcus, which gave moderately moist green colonies on blood agar and positive fermentation on lactose, salicin, and inulin. Capsules were not demonstrated. The second case was a young female with negative autopsy findings, no growth from the peritoneal cavity, and a pure culture from the heart's blood of a medium-sized, slightly elongated diplococcus, with occasional chains of four to six cocci and reactions on the media, as above. Capsular spaces were well seen in India ink preparations, but were not demonstrated by a variety of staining methods. The third case was from an adult female. The animal had given birth to young shortly before death. The organism was grown from the uterus along with the *Staphylococcus albus*. The morphology and reactions on media were similar to the above. No capsules could be demonstrated by staining methods. I am not convinced that these three organisms were pneumococci.

B. MUCOSUS CAPSULATUS GROUP. — The members of this group are rather infrequently found in guinea-pigs.

The organism described by Jensen under the name *B. centropunctatus* and which he isolated from guinea-pig manure probably belongs here.

The first case of spontaneous infection in a guinea-pig is that reported by R. Pfeiffer (1889). The case was very similar to many which I have had. The peritoneal exudate was glassy and slimy and made up almost entirely of bacilli and their slime, with remarkably few leucocytes. The cultures from the peritoneal exudate were typical and capsules were easily demonstrated. Coccoid forms were not seen. The characteristic point was the stickiness of the organ juices and blood. This organism is known as *B. capsulatus* Pfeiffer. In the same year Klein, in London, while working with an organism from pneumonia (*B. pneumosepticus*) had an epidemic of lung infection among his mice and guinea-pigs. Abel and Hallwachs believed this bacterium was closely related to *B. Friedländer*, but Klein himself said it was different, finding motility in his young cultures and not mentioning capsules nor a sticky character to the growth. The organism, which had been isolated from human cases had been used in experiments on animals, both by feeding and injection. The animals treated were in cages in the same room with the untreated (and spontaneously infected animals) and the organism recovered, as well

as the autopsy findings, were the same in all. I have found it impossible from the brief description given to classify this organism of Klein's. Chester and Migula place it in the colon-typhoid group. The organism isolated by Weaver in 1898, from a large female guinea-pig which died spontaneously after several days' illness with pneumonia, is probably a member of the *B. mucosus* group. Capsules are not mentioned, but the creamy white growth and the gas bubbles on potato are suggestive. He found it produced necrosis and cirrhosis of the liver in inoculated animals. Smith found *B. lactis aerogenes* once in a spontaneously dead guinea-pig.

The first definite epidemic among guinea-pigs due to *B. mucosus capsulatus* is that reported by Perkins (1901). Twenty-one animals were infected and two recovered. The autopsy findings were usually peritonitis and frequently a gaseous emphysema in certain of the organs. The animals which recovered were highly immune. Chrom has shown that guinea-pigs are particularly susceptible to infection with *B. Friedländer*.

My own results are in marked contrast to these few reports, since I have frequently found members of this group in guinea-pigs. *B. lactis aerogenes*, giving an extremely sticky growth, an easily-stained capsule, and acid and gas in dextrose, lactose, and saccharose, was found in forty-eight animals, twenty-eight times from heart's blood, twenty-four times from peritoneal fluid, seventeen times from the uterus, and eleven times from the pleural fluid. It was also isolated from the trachea six times, from extensive subcutaneous abscesses five times, twice from mastoid cells, and once each from abscesses of the liver, a peritoneal gland, and the mamma, as well as from the urine and kidney, pericardial fluid, lung, and spleen of single animals. In our series *B. acidi lactici* was obtained twice from the peritoneal fluid. The autopsy findings in these animals were most varied. As a rule, where septicemia was present all the cavities and the organs were markedly sticky.

In seventeen animals endometritis was the outstanding condition. *B. lactis aerogenes* was isolated in the majority of these animals from the uterus, the peritoneal cavity, and the heart's blood. It was usually not in pure culture from all these sources. In one case the heart's blood gave a pure culture of *Streptococcus pyogenes*, while the bacillus was alone isolated from the uterus and the peritoneal fluid. At

times the peritoneal fluid also contained *B. coli* or *Streptococcus fecalis* and from the uterus *Streptococcus pyogenes*, *Streptococcus fecalis*, and other bacteria were in certain cases found in mixed cultures with *B. lactis aerogenes*. The next most common conditions were found in the lungs and varied from congestion to old pneumonia with adherent pleurisy. In many of the cases listed under endometritis the lungs were also involved in the general infection. Cultures from pleural fluid, heart's blood, or trachea gave *B. lactis aerogenes*, but frequently streptococci were also isolated. One interesting case gave cultures of *B. lactis aerogenes* from the lung and trachea, while there were also isolated *Streptococcus pyogenes* and *B. bronchisepticus* from the lung and *Streptococcus fecalis* and *B. pyocyaneus* from the trachea. Abscesses were found ten times. In six of these very extensive phlegmonous and purulent infiltration of the subcutaneous tissues from the pubis to the neck was observed. Two were mammary abscesses. One was a large abscess in the mesenteric gland near the union of the appendix and the colon. Another was a large abscess in the liver with a small abscess about one-half inch from the tip of the right uterus. In most of these abscess cases a general infection had resulted, and although *B. lactis aerogenes* was usually isolated in pure culture from the body fluids, in some cases a mixed infection was found. One of the cases of mammary abscess developed a *Streptococcus pyogenes* septicemia. From the pleural fluid of another, *Streptococcus pyogenes* was isolated along with the bacillus, while from the peritoneal fluid of other cases *Streptococcus fecalis* was isolated twice, and from the heart's blood once.

The other interesting single cases from which this organism was isolated included a case of hemorrhagic peritonitis with a markedly enlarged spleen (5.5 x 2 x 1 centimeters) and a subcapsular hemorrhage greatly distending the posterior half of the organ. The bacteria were isolated from the heart's blood, peritoneal fluid, and spleen. A case with a blood-filled cyst in the liver gave similar bacteriological results. A small male pig (159 grams) showed dilated and

injected ureters and a small hard stone (12 milligrams in weight, 4 millimeters in diameter) in the bladder. *B. lactis aerogenes* was isolated from the urine and kidney.

Two guinea-pigs which had been sick for some time were brought to the laboratory. They both showed a disturbance in maintaining their equilibrium. In one purulent material was running from the right ear, in the other there was no discharge. Both animals were chloroformed and showed extensive double otitis media and mastoiditis. The cavities were filled with sticky pus and smears showed that all the organisms were encapsulated, many appearing in diplococoid form, resembling pneumococci. The growth gave pure cultures of *B. lactis aerogenes* in both cases. The first case was examined on March 15, the second April 25, 1916.

The number of cases showing infection with *B. lactis aerogenes* throughout the year was as follows: February nine, April nine, March eight, January seven, May five, December four, November three, June two, and October one.

B. BRONCHISEPTICUS. — The infection of guinea-pigs by this organism is of more than ordinary interest, since the recognition of *B. bronchisepticus* as a distinct bacterium is comparatively recent.

The bacillus *bronchisepticus* is a Gram negative, actively motile bacillus which from solid media appears extremely small, but from broth may be of fair size and even show short threads. It should be included in the group of *B. fecalis alkaligenes* (Petruschky) as it is very similar to this organism in its cultural and biological characters. The growth on potato which is used for differentiating *B. bronchisepticus* is not absolutely characteristic. The growth of *B. fecalis alkaligenes* on potato, which Petruschky also gave as the medium for differentiation of his organism, he described as a thick layer which browns the potato, and Terburgh described the growth on potato as giving a yellowish layer in twenty-four hours and on the following days a yellowish brown color. I have compared six strains of *B. fecalis alkaligenes* from human sources with four cultures of *B. bronchisepticus* from

guinea-pigs and could not find reliable differences, although the growth of the former is usually thinner and of a paler brown than the latter. The results in litmus milk, early and continued production of alkali, are practically the same in both. In broth *B. fecalis alkaligenes* was found to produce as a rule a surface growth, but this was not always the case. None of my strains of *B. bronchisepticus* have developed a film.

Südmersen described an organism which, undoubtedly, was *B. bronchisepticus*, giving a slight film on broth on the fifth and sixth day when undisturbed. Transfers from surface to surface increased this film production. He found this organism in the nose and it was often associated with *B. fluorescens liquefaciens* (probably *B. pyocyaneus* from his description). Berghaus identified *B. fecalis alkaligenes* with *B. fluorescens non-liquefaciens*, granting the loss of power to produce fluorescence. Klimenko also considered them closely related. Trincas and Olla described a fluorescent organism which they called *B. fecalis alkaligenes*, but which lost its chromogenic character after a few transfers. Theobald Smith believed that *B. bronchisepticus* approaches the *B. pyocyaneus* group, if it is assumed that in the course of special adaptation the former has lost the power of liquefying gelatin and producing pigment and, he might have added, of peptonizing milk.

My finding of *B. bronchisepticus* in the lung and *B. pyocyaneus* in the trachea of the same case is, therefore, of interest.

Briefly, the characteristics of *B. bronchisepticus* are as follows: It is a very small bacillus from agar slants and relatively small from broth. It is actively motile and is best isolated on solid media, the growth in the first twenty-four hours being characteristically slow. It gives a gradually increasing cloud in broth, with no scum but a precipitate, which becomes in time very stringy. There is no fermentation of any carbohydrate, no liquefaction of gelatin, serum, or casein, a marked alkaline production in all media (litmus milk), and a rich brown growth on potato.

The organism reported by Delbanco (1897), causing an epizootic of pseudotuberculosis among guinea-pigs, is described as non-motile, producing no gas in sugar media and no coagulation in milk. The earliest description of *B. bronchisepticus*, however, was by Tartakowsky (1899),

who failed to note the motility, but otherwise gave an excellent description of both the organism and the characteristic disease found in the lungs of one hundred and eighty-six pigs. Strada and Traina (1900) gave the name *Bacterium pneumoniae caviarum* to the organism they isolated. The epidemic was very extensive, over one hundred dying out of one lot and many more among other lots of guinea-pigs. Careful cultures were made from several animals, and they noted motility and described a slight scum on broth, otherwise their organism was similar to the above. Martini's cultures from two guinea-pigs, with the peculiar type of hepatization of the lungs, are probably the same organism, although he said that he found capsules. He gave to this organism the name *B. pulmonum glutinosus*. Südmersen (1905) gave a good description of an organism like *B. bronchisepticus*, which attacked the lungs and caused the death of many guinea-pigs. Selter isolated a motile bacillus from the lungs of guinea-pigs after blowing various kinds of dust into the trachea, as well as from the lungs of untreated animals. He used the name *B. cavisepticum mobilis* for this organism which, from his description, is clearly *B. bronchisepticus*. In 1908 Klimenko described *Bacterium mariense* (nov. spec.) as a new alkali producer. It was recovered from the spleen and blood of an apparently healthy guinea-pig. This organism gave a fine scum on broth after seven or ten days, and the growth on potato was like *B. coli*, but not as luxuriant. Otherwise the characters of this bacillus are the same as *B. bronchisepticus*. Klimenko further differentiated it from *B. fecalis alkaligenes* and *B. fluorescens non-liquefaciens* by means of agglutination tests.

M'Gowan in 1910 first used the name *B. bronchisepticus* for the organism he described, and which he had isolated from a variety of animals, including three guinea-pigs which showed symptoms of distemper and four without symptoms. The organism was found in the trachea, often in pure culture. Ferry (1912) obtained this organism from sixty-eight guinea-pigs out of seventy-five examined. He isolated it from the trachea sixty-three times (fifty-six in pure culture), from the lung thirty-one, from the liver twenty-two, and from the heart's blood seventeen times. None of these animals showed any signs of an infection of the nasal cavities or eyes. In a later communication (1914) he gave the symptoms of the infection by this organism in guinea-pigs, and considered the reactions in litmus milk and on potato as characteristic. The strain of *B. fecalis alkaligenes* mentioned by him, and which gave a distinct acid reaction in litmus milk, was certainly misnamed.

One of the fullest and best contributions on the *B. bronchisepticus* infection in guinea-pigs is that of Theobald Smith (1913). He has called attention to the frequency of chronic infection by this organism, and gave an excellent description of the lung conditions and of the characters of the organism. He considered it the primary infection in his cases of pneumonia, and believed the pneumococcus infection was a secondary one. Ferry in 1915 showed that this bacillus may pass through the ordinary

filters, and this finding may explain the diseases of guinea-pigs, reported as due to filterable viruses by Petrie and O'Brien, Römer, Gasperi, and Sangiorgi. Smith, however, did not find it filterable.

In my studies I have not found *B. bronchisepticus* very often, having isolated it from only twelve guinea-pigs (six times from the lungs, ten times from the trachea, and once from the pleural fluid). Cultures were made from the trachea of thirty-eight animals and in thirteen no growth occurred. From the cases with positive cultures and which did not yield *B. bronchisepticus*, various other organisms were recovered. These tracheal cultures were made just above the division of the bronchi. The surface was seared, opened with sterile scissors and a platinum spatula inserted. A fairly large amount of material was used. Plain agar is necessary to grow *B. bronchisepticus* in the primary culture, as it was not recovered from the serum broth when duplicate cultures were made. This is probably due to the rather strict aërobic character of the organism. Gaechtgens also advised the use of plain agar to isolate *B. fecalis alkaligenes*. The distribution of *B. bronchisepticus* infection during the year, shows five in April, three in March, two in February, and one each in January and November.

B. PARATYPHOSUS GROUP. — Members of this group have been frequently reported as infecting guinea-pigs.

The early reports of Malassez and Vignal, Chantemesse, Charrin and Rogers, Dor and A. Pfeiffer on pseudotuberculosis do not contain sufficiently full descriptions of the bacteria to positively identify them. However, we may conclude from the later reports of others, that some of these earlier cases were possibly due to members of this group.

Schantyr (1891) described a small non-spore bearing motile bacillus which gave gas in gelatin, but no liquefaction, a yellowish growth on potato and was Gram positive, which he believed was the cause of puerperal fever in guinea-pigs. The disease occurred in May, June, and July, and attacked females about the time of parturition or after abortion, as well as new-born animals. The latter died with an acute septicemia. The mother pigs showed, at autopsy, purulent metritis, ulcerative vaginitis, enlarged spleen, fatty liver, and other unimportant changes. The smears from the various parts showed masses of small bacilli. Cocci were also noted in smears from the lung, uterus, and vagina. Twenty-three

pure cultures were studied. It is unfortunate that this bacillus cannot be identified. This author quotes Semmer as having had similar outbreaks during the five previous years, and four epizootics of pneumonia, all due to a small characteristic bacillus. It is possible that it is the same organism which Carter found in the infectious metritis, and if so, it is a member of this group.

The report of Klein (1892), in which, after injecting a guinea-pig with *Staphylococcus pyogenes aureus*, he recovered an actively motile bacillus which gave no coagulation of milk and gas bubbles in gelatin, is important as indicating the danger of secondary invasion in experimental animals. This organism may have belonged to this group.

Pseudotuberculosis is the most characteristic disease produced by the paratyphoid group of bacilli. *B. paratyphosus*, *B. enteritidis* (Gaertner), *B. aertryck*, and the alpha type of *B. paratyphosus* (Kirch), are all reported as the cause of this condition.

Theobald Smith first established the importance of the paratyphoid bacilli in guinea-pig infections. In 1894 he gave the first evidence that pseudotuberculosis of guinea-pigs was due to members of the hog cholera group; in 1897 he completely identified the bacillus from a spontaneous case of pseudotuberculosis as belonging to this group; and finally, in 1903, he showed the agglutination affinities of these bacilli and included Carter's bacillus of infectious endometritis in guinea-pigs. This latter organism showed no appreciable difference from the members of this group isolated from cases of pseudotuberculosis.

Durham (1898-1899) believed, from experimental evidence with *B. enteritidis* (Gaertner), that pseudotuberculosis of guinea-pigs was transmitted by means of food. The finding of paratyphoid bacilli in the intestines by Morgan and Marshall, as well as the "carrier" cases reported by O'Brien, and the production of the disease by feeding, as shown by Dieterlen and Kirch, would give weight to this suggestion. There are many references in the literature to pseudotuberculosis caused by these organisms since Theobald Smith's work, the most important being MacConkey (1905), Wherry (1908), Eckersdorff (1908), Dieterlen (1909), De Basi (1909), Petrie and O'Brien (1910), Bainbridge and O'Brien (1911), and others. It will be unnecessary to more than briefly summarize the more important findings of these authors. MacConkey found an organism indistinguishable from *B. enteritidis* in four animals dying during a small epizootic of this disease. From the heart's blood of two guinea-pigs injected with pure cultures of *B. mallei* and *B. typhosus* respectively, the same organism was isolated. Wherry named his organism *B. pestis caviæ*, but found it identical with Carter's bacillus which, as noted above, is *B. paratyphosus*. The epizootic Eckersdorff reported was very extensive, one hundred animals being killed. The chief interest in Dieterlen's cases was that the disease was found after inoculation with suspicious tuberculous material in four animals. Petrie and O'Brien reported an epizootic which destroyed all but twenty-one of a stock of five hundred guinea-pigs. Five out of nine of those living were found to be

carriers of the bacillus which O'Brien showed by agglutination tests stood nearest to *B. aertryck*. These authors, however, believed the disease was due to a filterable virus. Bainbridge and O'Brien in two further epizootics had a mortality rate of between sixty and seventy per cent and found besides the characteristics of pseudotuberculosis, abscesses in the mesenteric glands and peritonitis or pericarditis in some of their animals.

The fact that bacilli of this group have been recovered after the guinea-pigs have been treated in various ways as shown by the results of Smallmann (1903), Klein (1905), MacConkey (1905), and Bofinger (1911), and the finding of these organisms in the organs of healthy animals by Ford, Petrie, and O'Brien, Bofinger and others, should be remembered in experimental work. Smallmann in an article on the possible interrelationship between the typhoid and paratyphoid bacilli found the latter organism in guinea-pigs after they had been injected with killed typhoid bacilli or their extracts. Klein after injecting the precipitate from three hundred cubic centimeter samples of milk found, in the guinea-pigs used for the experiments, nodules in the spleen, isolated *B. enteritidis* (Gaertner) and believed it came from the milk. MacConkey is quoted above. Bofinger isolated the paratyphoid bacillus from guinea-pigs which had been injected with suspicious sputa. He was, however, unable to grow this organism from the original sputa and he recognized the danger of misinterpretation after he had isolated the same bacillus from healthy guinea-pigs which had been killed for complement. Ford (1900) isolated the paratyphoid bacillus from the liver and kidney of untreated guinea-pigs, and Petrie and O'Brien also found this organism from a number of healthy animals. It is interesting to note that in Boxmeyer's epizootic of streptococcus infection the only other organism found was a member of the hog cholera group and the animals from which it was isolated showed the characteristic lesions in the liver and spleen. A number of reports in which the description of the bacteria is incomplete or differs from that of the paratyphoid bacillus I will now shortly review.

Lubenau (1901) described an organism from a guinea-pig septicemia which did not coagulate milk and produced gas in broth. It possibly belonged to this group. Lochmann's *B. caseolyticus* may be classed here as it is included in the paratyphoid group by Uhlenhuth and Hubener. It gave a brownish growth on potato, alkaline in milk, and produced gas in dextrose, lactose, and saccharose, as well as in media without the addition of the carbohydrates. This latter observation would indicate that the basic media contained fermentable substances attacked by this organism. The variety of colon bacillus reported by Kovářík which did not coagulate milk and considered by him as identical with *B. caseolyticus*, was recovered from an epizootic among guinea-pigs. Lochmann found his organism in four guinea-pigs which had been injected with pure cultures of tubercle bacillus and Kovářík found his in an epizootic disease resembling pseudotuberculosis, accompanied with diarrhea and a type of paralysis of the hind legs. The organism isolated by Cagnetto (1905) from a pseudotuberculosis of guinea-pigs is not easily classified. It was

actively motile, variable in size, Gram negative, gave a slight pellicle on broth, slow growth on agar, no liquefaction of gelatin, no change in the reaction in milk, but a partial coagulation, acid, but no gas in dextrose, and a thin glanders-like growth on potato, but never a brown color. He applied the name *Bacterium pseudotuberculare orchitophlogenes*, since it gave orchitis and vaginitis in injected guinea-pigs, and he considered it closely related to the pseudoglanders bacillus.

It is seen from this review that the spontaneous infection of guinea-pigs with bacteria of the paratyphoid group is very common in all parts of the world. It is therefore quite remarkable that I have not isolated any organisms which I felt certain belonged to this group in my series of animals, nor have I met a disease of guinea-pigs simulating pseudotuberculosis. McMeans at Bellevue Hospital, New York, however, isolated from three spontaneously dead guinea-pigs paratyphoid-like organisms during the last year.

B. COLI GROUP. — There is not much evidence that the colon bacillus is of importance, excepting as a secondary invader, in infections of guinea-pigs.

Andrewes (1892-1893) reported the isolation of a colon bacillus from a guinea-pig which had been injected with *B. prodigiosus*. The peritoneal fluid was passed from animal to animal, and the plates made at the same time from the fluid of each animal showed the proportion of the injected bacillus and the invader. It required five animal passages before the invading colon bacillus was the predominant organism on the plates. Weaver (1897-1899) isolated from a guinea-pig a colon-like bacillus, which he used for the experimental production of hepatic cirrhosis. Strada and Traina believed the finding of *B. coli* in their cases was due to post-mortem invasion. Galli-Valerio (1901) described a pseudotuberculosis bacillus which coagulated milk. He probably had either a mixed culture, with a lactose fermenter or a true colon bacillus. Small hemorrhages in the walls of the stomach are reported by Harris as resulting from an intraperitoneal injection of a strain of *B. coli communis* isolated from the intestines of a guinea-pig. Eyre also found *B. coli* in the intestines of guinea-pigs. Schwarz (1906) isolated *B. coli communis* from necrotic areas in the liver of a spontaneously dead guinea-pig. This organism produced a toxin which was highly resistant to heat. From pseudotuberculosis in a guinea-pig, Simon isolated an organism giving the cultural characters of *B. coli communior*. Theobald Smith has found the colon bacillus a number of times in his guinea-pigs. Arlo found it once

in the lungs of healthy guinea-pigs. *B. caseolyticus* was considered by Lochmann to be more closely allied to *B. coli communis* than to any other organism.

I have isolated varieties of the colon bacillus twenty-three times, seventeen times from the peritoneal fluid, four times from heart's blood, three times from trachea, twice from pleural fluid and uterus, and once from the eye. The evidence in all these cases pointed to its being a secondary invader. It was pure only five times from the peritoneum, in three of these definite infection with various organisms was present in other parts. The mixtures usually included either secondary streptococci or the organism isolated from other definitely-infected parts, such as *Streptococcus pyogenes*, or the *B. lactis aerogenes*.

ACID-FAST BACTERIA. — On account of the general use of guinea-pigs in testing for the tubercle bacillus, it is important to realize that they are extremely susceptible to tuberculosis. In this connection it should be remembered that pseudotuberculosis may be easily mistaken for tuberculosis by the inexperienced.

Griffith in the Report of the Royal Commission on Tuberculosis gave numerous cases of spontaneous tuberculosis in guinea-pigs. Fürst had two cases listed in his table as tuberculosis, one in the lung and the other in lung, liver, and spleen. Feyerabend reported a spontaneous infection in an animal which had received milk from a tuberculous goat. Bartel and Spieler demonstrated the infection of guinea-pigs kept in the room of a tuberculous patient, and also had three spontaneous infections in older animals. These authors also recorded the death of guinea-pigs from pneumonia among the younger animals, and especially in the spring and fall, as well as from pleurisy, peritonitis, and other diseases. The bacteriology is not given for these latter cases. The finding of smegma-like bacilli by Cowie has already been noted.

PASTEURELLA GROUP. — Guinea-pigs are apparently relatively non-susceptible to organism of this group. The bacteria of this group are non-motile, Gram negative cocco-bacilli, staining more deeply at the ends and often pleomorphic. They are non-spore bearers, do not liquefy gelatin nor coagulate milk, give no visible growth on potato, are

primarily aërobic, and in many ways resemble the plague bacillus. They are differentiated from the latter by the results of animal injections and certain carbohydrate fermentation tests. It is important to remember that natural plague has been observed in guinea-pigs in India and elsewhere.

Phisalix (1901) described an organism of this group as the cause of septicemia in guinea-pigs. The bacterium, however, gave a luxuriant growth on potato and a slight acid in dextrose and milk and cannot be very definitely classified. The disease resulted in a fibropurulent deposit on the peritoneal organs and hyperemia, but never hepatization of the lungs. In 1906 Byloff reported an extensive pest-like epizootic which attacked guinea-pigs. The important finding at autopsy was the presence of tumors of various sizes in the pelvic cavity. In five, which showed paralysis of the hind legs, there were greatly enlarged glands near the plexi of the nerves. The tumors appeared to be in the lymph channels and the folds of the mesentery; they were of various sizes, and a few were found to be caseous. Small nodules were also found on the liver and spleen and more rarely on the pancreas. The organism isolated from various parts conforms to the characters of the members of the hemorrhagic septicemia group in which the author placed it. He gave it the name *B. pestis intestinalis caviæ cob*, and believed it to be the same organism which Zlatogoroff had previously found to be closely allied to *B. pestis*. Laven (1910) described an organism of this group obtained from a rabbit and which he considered identical with an organism formerly isolated from guinea-pigs which had died with purulent peritonitis and beginning pneumonia. The two organisms were probably not the same. Zeiss (1914) described a keratitis of the guinea-pig and obtained from the pus a pure culture of a bacillus of the hemorrhagic septicemia group.

In my animals I have not had an infection with bacteria of this group.

DIPHTHERIA-LIKE ORGANISMS.

Klein showed at the International Congress of Hygiene in 1891 cultures of diphtheria bacilli which he had grown from man, cow, and guinea-pig. Graham-Smith reported the isolation of *B. xerosis canis* from the eyes of fourteen guinea-pigs. Teacher and Burton (1914-1915) isolated a diphtheroid bacillus from the yolk sac of pregnant guinea-pigs and considered it the cause of an outbreak of infectious abortion. These authors quoted MacFadyean as saying that Bang's bacillus of abortion has given rise to epizootics of abortion in his stock of guinea-pigs. Surface (1912) found amboceptors for the abortion bacillus in twenty-nine from forty-three

guinea-pigs. He believed these were infected from the inoculated animals and was able to recover the organism twice. Smith has noted several points of resemblance between *B. abortus* and *B. bronchisepticus*.

I have encountered bacteria belonging to this most undetermined group of the so-called pseudodiphtheria bacilli in six guinea-pigs. Twice from the heart's blood and pleural cavity and once from a subcutaneous abscess and the trachea. In most of these it was not in pure culture. In the heart's blood of one pregnant pig it was pure and the only organism recovered. In another it was pure in the pleural fluid of a pig dead after injection with killed *B. coli*.

B. PYOCYANEUS.

Kovářzik said that Phisalix had a small outbreak among guinea-pigs from which *B. pyocyaneus* was isolated. Südmersen found *B. pyocyaneus* named by him *B. fluorescens liquefaciens*, associated with a bacillus corresponding to *B. bronchisepticus* in the nasal cavity of his animals.

I have isolated the *B. pyocyaneus* once from the trachea, once from the heart's blood with other organisms, and once from the trachea in a case giving *B. bronchisepticus* from the lung.

OTHER MICROÖRGANISMS. — Spore-bearing bacilli have been found by a few.

Eberth (1885) illustrated a spore-bearing bacillus from pseudotuberculosis and Dor found a spore on the bacillus studied by him from the same disease. Ford found them in the liver and kidney, and Selter found them in the lungs of healthy pigs.

I have isolated only two spore-bearing organisms, once from the lung and once from the trachea. *B. proteus* has been reported as a contaminating organism by a few writers (Wagner, Strada, and Traina). I have found it once in the trachea and once in the peritoneum.

Coccidia are mentioned by Strada and Traina as infecting guinea-pigs, and Pianese found a sporozoarium of genus coccidia in the kidneys of many guinea-pigs. Griffith also reported coccidia in a number of pigs.

Ameba cobayæ is reported by Walker and several are listed as coming from guinea-pigs by Hassall. Perroncito reported an intestinal disease in guinea-pigs with many deaths which he believed was caused by two forms of *cercomonas*. Galli-Valerio reported an epizootic with a high mortality, due to *Trichomonas caviæ* Dav. Hyperemia of the large intestine, was seen at autopsy and the contents contained many of these flagellates. De Gasperi accidentally found spirochetes in the blood of guinea-pigs, while Sangiorgi found a similar spirochete, named by him *Spiroschaudinia caviæ*, in liver lesions of a guinea-pig after injection of the virus of guinea-pig plague. Horta reported two cases of primary infection of the guinea-pig with *trichophyton gypseum asteroides*. Spontaneous infection of guinea-pigs with *saccharomyces niger* is reported by Maffucci and Sirleo (1895 and 1897). Kurloff bodies were shown by Knowles and Acton to be especially well seen in the leucocytes of guinea-pigs. These were not considered parasites. The leucocytozoon of Ross is also probably not a parasite.

Diseases due to a filterable virus have been reported by Petrie and O'Brien (1910), Römer (1911), and de Gasperi and Sangiorgi (1913). Petrie and O'Brien reported an epizootic in which a bacillus of the paratyphoid group was found in association. They carried out numerous filtration experiments and concluded the disease was due to a filter-passer. Römer had a disease of guinea-pigs which resembled infantile paralysis in man. He considered it due to a filterable virus which he constantly obtained from the brain, spinal cord and glands, and at times from the liver and spleen. It was not found in the lungs, kidneys, urine, bile, or blood. The virus was resistant to fifty per cent glycerine, was invisible, could not be cultivated and was filterable. De Gasperi and Sangiorgi had an epizootic and lost fifty-six guinea-pigs. The animals became emaciated, had difficulty in respiration, and developed marked nervous symptoms before death. The autopsy examinations showed marked congestion in the viscera and in the medulla oblongata. The virus which they considered the cause of this disease was obtained from the brain, but was also present in the circulating blood, the feces, urine, and bile, was resistant to glycerine, putrefactive changes, and drying. It was destroyed at a temperature of 70–72° in one hour.

There are a number of other diseases of guinea-pigs recorded in the literature, such as diseases of the cornea by Sommer, but no bacteriology is given. The work of Holst (1907), Fürst (1912), and Holst and Frölich (1912), demonstrated the importance of feeding in the production of scurvy, and also that certain tissues and regions of the body are definitely damaged after various types of feeding. Among the conditions found by these investigators were abscesses, pneumonia, pleurisy, peritonitis, perforation of

the duodenum, petechial hemorrhages in the skin and the muscles, often about the knee, hyperemia of the ribs, and softening of the epiphyses of the long bones. Such animals would have a definite lowered resistance in these areas and bacterial infection would follow spontaneously as shown by Jackson and Moody (1915), who recovered non-hemolytic streptococci from similar lesions in experimental scurvy. Theobald Smith (1905) has reported wide variations in the susceptibility of guinea-pigs to diphtheria toxin and considered it due to insufficient food and unsatisfactory environment. Rosenau and Anderson (1906) found gastric ulcer in many hundreds of guinea-pigs after injection with diphtheria toxin. Werz (1907), in experiments on the bacteriology of holy water, used guinea-pigs and supposedly recovered many organisms. Whether the bacteria isolated were actually in the water is open to question. Seidelin (1915) found lesions in guinea-pigs experimentally infected with yellow fever which are quite similar to those occurring spontaneously. He did not consider them characteristic for the disease in these animals.* Malignant tumors are extremely rare in guinea-pigs. Sternberg (1913) reported an adenocarcinoma of the mammary glands and Jones (1916) found a similar tumor which was transplantable.†

CONFUSING EXPERIMENTS. — All the authors quoted have not recognized the spontaneous nature of many of these infections nor the confusion liable to arise where the experimental animal is previously infected, or where invasion and infection from the animal follows the treatment or the inoculation in the experiment.

Galli-Valerio warned against the use of guinea-pigs without a full knowledge of the pseudotuberculosis infection. Such results as Bofinger's

* Seidelin further described what he believed to be division forms of *Paraplasma flavigenum* in the red cells of treated guinea-pigs. Wenyon and Low (*Jour. Trop. Med. and Hygiene*, 1915, xvii, 369) found bodies indistinguishable from these in normal animals.

† In a recent article Murray (*Jour. of Path. and Bact.*, 1916, xx, 260) reported a transplantable sarcoma of the guinea-pig.

recovering paratyphoid bacilli after injections of sputum (he recognized the error), Klein after milk injections, and those of numerous investigators after suspicious tuberculous material (Chantemesse, Dieterlen, Malassez and Vignal) and others (Poppe) might easily lead to misinterpretations. The paratyphoid organism has also been recovered after injections of *B. mallei* and *B. typhosus* (MacConkey), *B. mallei* (Pfeiffer) and *B. typhosus* by Smallmann, who apparently believed that the two were closely related.

The isolation of pneumococcus from guinea-pigs following inoculation with various bacteria by Selter was not misinterpreted, since he also found it in untreated animals.

The guinea-pig is a highly susceptible animal and frequently becomes infected from other experimental animals. Horne reported a streptococcus infection, spreading from the lemmings of the laboratory to his guinea-pigs, Weber an epizootic of streptococcus infection among his rabbits, followed by an outbreak among his guinea-pigs, and Christiansen an epizootic of pneumococcus infection from infected calves, secondarily attacking the guinea-pigs. Ungermann while working experimentally with the pneumococcus found a spontaneous infection with this organism in a guinea-pig. Klein had a spontaneous epizootic among the stock guinea-pigs, due to an infection with the same organism used to inoculate animals. These treated animals were in the same room as the untreated, but in different cages. Südmersen reported an infectious pneumonia of rabbits due to a bacillus (*B. bronchisepticus*), which secondarily attacked the guinea-pigs. Such infections may, however, come from a common source and be due to environmental changes. Feyerabend's and Bartel and Spiel-er's cases of spontaneous tuberculosis are of importance. Surface believed his guinea-pigs were infected with *B. abortus* from his inoculated animals. Nicolle and Conseil reported that guinea-pigs, bought from a goat herder in Malta, were infected with *Micrococcus melitensis*, and many other reports indicate the liability to error from such spontaneously-infected animals. The animals may become infected in many ways, and more especially through food, as is believed to be the case, particularly, in the paratyphoid infections.

There is one striking conclusion to be drawn from the results here given. In different localities the organisms infecting guinea-pigs differ widely. Epizootics due to a certain organism are found in certain localities and not in others. It is remarkable, for example, that in my series I have not met with either the pneumococcus or the paratyphoid-like bacilli, although natural infections by these are not uncommon in all parts of the world.

It is important to determine the bacteria which spontaneously infect animals in different localities, and the various

conditions spontaneously produced by these bacteria, as, on the one hand, they are liable to lead to confusion in experiments, and, on the other, they serve to indicate the natural susceptibilities of different tissues of particular groups of animals. Seasonal variations, in both the prevalence of certain types of bacteria and the diseases and tissue changes they bring about, are also of the greatest importance, as is indicated in the reports quoted above.

The presence of carrier cases with different bacteria has been frequently noted, and the work of Huet has demonstrated that male guinea-pigs may carry infecting organisms in the seminal vesicles after recovery from a variety of experimental infections. It is also to be noted that latent infection is not uncommon, and that chronically-infected cases frequently spread disease. The mechanical transfer of infection by the male is to be considered, especially in puerperal infections, as it is well known that the male quite frequently has intercourse very shortly after the female is delivered.

From the facts brought together in this paper it is evident that guinea-pigs are frequently subject to a great variety of spontaneous infections, and it is, therefore, necessary to be familiar with these before using such animals for experimental purposes. The literature is full of similar infections in other laboratory animals, such as rabbits, cats, dogs, mice, rats, in fact in practically all animals, and it is advisable that all spontaneous infections in these animals be promptly reported in order to increase our knowledge and as a help in preventing misinterpretations from experimental work.

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THE ACCUMULATION OF ANISOTROPIC FATS IN INTERSTITIAL CELLS OF THE KIDNEY

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THE ACCUMULATION OF ANISOTROPIC FATS IN
INTERSTITIAL CELLS OF THE KIDNEY.*

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Although the subject of artificial feeding of cholesterin has been extensively investigated of late, we have seen no record of the effect of such experiments upon the kidney. While engaged in experiments of administering cholesterin to rabbits we noted certain definite changes in the interstitial tissue of the renal medulla.

We are familiar with the tissue changes which have been described in the experimental feeding of lipoids. The establishment of cholesterin, as the causative factor of the changes produced in feeding experiments, was proved by Stuckey and Wesselkin. Anitschkow has demonstrated the development of fatty plaques in the aorta of cholesterin-fed rabbits. Rothschild showed the relation of the Kupffer cells of the liver and Sternberg the function of the adrenal in cholesterin metabolism. Chalатов noted the laying down of cholesterin-esters in the liver, while Aschoff and Landau have called attention to the importance of endothelial tissue in dealing with cholesterin materials. We found in our own work, during a study of the nature of the lesion produced by cholesterin in the aorta of rabbits, organic response similar to that reported by other authors, and, in addition, noted that the kidneys of some of our animals showed a change which was unfamiliar to us.

The animals were daily fed by a stomach tube with cholesterin, some animals receiving the cholesterin in olive oil, while a sodium oleate cholesterin emulsion was fed to others. Five of the animals were given a daily dose of cholesterin varying from .28 to .56 gram. Two of these

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rabbits, R. 2 and R. 3, showed no macroscopical evidence of change in the aorta while the kidneys were affected. Animals R. 4, R. 6, and R. 9 had developed fatty plaques in their aortas as well as changes in the kidneys. Rabbit 9 exhibited the most marked production of fatty intimal change in aorta and also the most intense studding over of the medullary portion of its kidneys by small white areas. Another rabbit, R. 12, receiving seventy-five grams of cholesterin in nineteen days showed no change in its aorta or kidneys in the gross. Although the kidneys of R. 12 were negative in the gross there was microscopic evidence of change simulating the cellular reaction noted in the kidneys of our other animals.

Animal.	Days Treated.	Daily Dose of Cholesterin.	Total Cholesterin.	Fatty Streaks in Aorta.	Change in Medulla of Kidney.
<i>Cholesterin Olive Oil.</i>					
R. 2 . . .	57	0.28	8.12	None.	Present.
R. 3 . . .	61	0.28	15.96	"	"
R. 4 . . .	86	0.28-0.56	31.92	Well developed.	Well developed.
R. 6 . . .	108	0.28-0.56	44.24	" "	" "
<i>Sodium Oleate Cholesterin.</i>					
R. 9 . . .	115	0.56	61.6	Very marked.	Very marked.
R. 12 . .	19	3.94	75	None.	Present.

Macroscopically the reaction was localized in the pyramids of the renal medulla. When the organ was sectioned from pole to pole yellowish white streaks marked the pyramids. These were arranged radially below the intermediate zone. They bulged upon the cut surface and their direction was that of the collecting tubules. On cross section of the organ these areas were seen as milk-white dots scattered through the tissue.

Microscopically these white areas were confined to the interstitial tissue of the pyramids. The earliest alterations were noted in the kidneys of R. 12. There was a beginning proliferation of the lining cells of the small capillaries. As

each successive animal received more cholesterin over a longer period of time the proliferation of the interstitial cells became more pronounced. The most advanced lesions were observed in rabbit R. 9. Paraffin sections showed the production of islands of cells growing between the tubules and compressing them. The cells in these areas were large and irregular, their shape depending upon their close arrangement. Some were round, while again others were elongated with flattened sides. Their protoplasm was swollen and made up of a delicate network, between the meshes of which there were small spaces. At times quite large vacuoles were present in the protoplasm. The nuclei as a rule were round, quite small, and deeply stained. However, in some cells the nuclei were larger and somewhat vesicular. In most instances the cells were surrounded by well-defined membranes, although at times several cells were fused together and the individual cell membranes could not be made out. Between the large cells red blood corpuscles were frequently seen. In fact, the structure had the appearance of small capillary channels with a proliferation of the lining endothelium. The renal tubules in the vicinity of these areas were easily recognized and were bounded by intact basement membranes (Mallory). Some of the tubules were dilated and lined by flattened cells. The epithelial cells of these tubules commonly presented a granular hydropic protoplasm.

In the earlier stages of proliferation of the interstitial cells the Sudan and hematoxylin staining brought out features which were not observed in the paraffin preparations. Throughout the interstitial tissue of the medulla, the lining endothelial cells of the small capillaries contained small Sudan globules. In one animal (R. 12) the endothelial cells of the glomerular tufts contained Sudan material with a fair amount of the same substance in the lining cells of the small interstitial vessels. When the proliferation of the lining cells of the capillaries was advanced, so that islands of cells were formed, the reaction resembled the accumulation of cells within the intimal fatty plaques of the aorta. The cells were large and swollen, and their bodies filled with Sudan globules

giving them a beaded appearance (foam cell). Where the cell membranes were not distinct, a large fatty mass was formed through which the deeply stained nuclei were seen. Occasionally an overloaded cell liberated some of its fatty content into the surrounding tissue. The fat within the foam cells was anisotropic.

In the lining cells of some of the tubules, close to the proliferation in the interstitial tissue of the pyramids, small globular Sudan material was observed. The desquamated cells lying within the tubules frequently contained Sudan stained globules. However, in comparison with the amount of fat locally stored by the endothelial islands, the cells of the tubules were remarkably free.

Aside from the above interstitial reaction there was another reaction which occurred in three of the kidneys, R. 4, R. 6, and R. 9. This reaction consisted of a proliferation of cells in the cortex. These cells were about the size of the lining cells of the convoluted tubules. They had a homogeneous cloudy protoplasm and centrally placed round nuclei. The direct relation of these cells to the tubules could be established by the use of Mallory's stain. It appeared as though the lining cells, by proliferation, had distorted and dilated the tubule. In one area tubular proliferation had occurred about a glomerulus. In the Sudan and hematoxylin sections a number of the above described cells contained granular Sudan material.

A proliferation of cells within the interstitial tissue of the pyramids of the kidney is quite a unique condition. From the location of the islands of cells scattered through the interstitial tissue of the pyramids, particularly in its upper half, attention is drawn to the possible relation of this proliferation with the lining cells of the medullary capillaries descending from the arcuate vessels. In our observation of the earliest response the lining cells of the small arterioles in this region showed some proliferation and already contained fat. In our subsequent animals where sufficient time for the proliferation of cells of the arterioles had elapsed, islands of cells were formed. The animals, R. 4, R. 6, and

R. 9, which presented the most marked lesion in the kidney, also showed well developed fatty plaques in the aorta. Again, in animals R. 2, R. 3, and R. 12, where there were no fatty plaques in the aorta, there were other evidences of a similar reaction. The response was also present in the Kupffer cells of the liver, the lining cells of the small arterioles of the heart, the arterial sinuses of the spleen and the arteriæ rectæ of the kidney. Thus it would seem that the endothelium of the more delicate vessels was first affected. When a considerable time is afforded to the experiments the endothelial tissues respond to the continued hypercholesterinemia by the development of areas which can be recognized in the gross, just as the appearance of the fatty plaques in the aorta. We are of the opinion that the reaction observed in the interstitial tissue of the pyramids of the kidneys was a response of the endothelial cells of the arteriæ rectæ and their capillaries. Furthermore, this proliferation has occurred as a part of a general response of endothelial tissues to the excess of cholesterol compounds in the blood.

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Occurrence of Arteritis in Meningitis

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THE OCCURRENCE OF ARTERITIS IN MENINGITIS.

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THE part played by inflammation in alterations of the intima of arteries is still an unsettled question. This is illustrated by the wide diversity of opinion which prevails among many of the foremost investigators. Some (Adami, Thoma, Jores) are of the opinion that the cases of chronic thickening of the intima are usually dependent upon mechanical disturbances, while others (Klotz, Hansen, Simnitsky) have shown that similar intimal changes may be found in the arteries of young individuals who had suffered from acute infectious diseases. The adherents of the mechanical theory hold that the intimal thickenings are commonly secondary to a medial weakening.

Rokitansky, an early investigator of nodular thickenings of the intima, held that they were the result of an organization of materials deposited from the blood stream upon the intimal surface of the vessel. He was of the opinion that the intima, being a non-vascular structure, was not capable of undergoing an inflammatory change. The error of this view was clearly shown by Virchow, who described "endarteritis chronic deformans" as a parenchymatous inflammation of the intima, with active proliferation of the cellular elements and at the same time a metamorphosis of the intercellular substance.

Klotz has pointed out that the nodular aorta is the result of repeated insults telling upon the intima alone, and that the thickenings may be entirely proliferative, representing a chronic inflammatory production. Concerning the nodular thickenings about the intercostal arteries, he finds the reaction an inflammatory one and accompanied by progressive as well as degenerative changes in the tissues of the intima.

Again, we find that an endarteritis has been induced experimentally by the employment of various microorganisms. Klotz, who injected rabbits intravenously with *B. typhi* and streptococci of low virulence, obtained warty thickenings of the first part of

the pulmonary and ascending limb of the aorta. Microscopically, these areas showed a fatty degeneration of the subendothelial tissue, with much connective tissue advancing into the degenerated area. Saltykow has noted similar results by the intravenous injection of staphylococci.

Boinet and Romary by the employment of different organisms (*B. typhi*, *B. coli*, streptococci, staphylococci, *B. anthrax*, tubercle bacillus, tetanus, and diphtheria) observed that tiny yellow plaques were produced in the aorta. Microscopically, there was a cellular infiltration in the intima, at times extending into the remaining layers, but most frequently almost entirely isolated to the adventitia around the vasa vasorum. Pernice studied the effect of the *Staphylococcus aureus* and found an inflammatory reaction in all three coats, consisting mostly of a round-celled infiltration, and in the most severe cases the inner layers of the intima contained large cellular elements.

Sumikawa, who irritated the vessels by painting them with turpentine or silver nitrate or again infected them with bacteria, demonstrated that the vessels so treated showed an inflammatory reaction in all the coats or else in the intima alone. It has been shown by Stumpf that in cases of infection by direct continuity the process proceeds from the adventitia inward and may advance to the intima, which may in turn develop a verrucose endarteritis.

Thus we find that not only is there a true endarteritic thickening of inflammatory origin, but also that the intima is capable of showing a definite inflammatory reaction with the presence of a cellular infiltration. However, the mode by which this reaction takes place, the relation of this inflammation to the different structures within the coats, is still open to discussion.

Koester believed that the primary lesion of arteriosclerosis was an inflammation of the media. There is, he said, a chronic inflammation following the vasa vasorum from the adventitia into the media while the intimal reaction is only secondary to this. He further found that each plaque of endarteritis had one or more complementary areas of mesarteritis beneath it. In regard to the above discussion by Koester, we find the following noted in the recent work of Klotz. "The simultaneous presence of a small-celled infiltration in the vicinity of the vasa vasorum and the intima may be observed during the acute stages, but we have not been able to demonstrate a constant relation between them in the arterial wall. The localization of one or other in the artery is not always accompanied by a similar reaction in the other arterial coat opposite to it. In other words, though an inflammatory reaction may be demonstrated about the vasa vasorum in the adventitia and outer portion of the media a similar process may not be opposite to this in the intima. Moreover, where the simultaneous presence of a cellular infiltration has been observed in the inner and outer coats

of the artery there has always appeared a strip of media adjacent to the intima which was uninvolved in the inflammatory process. It would, therefore, appear that these reactions are individual, though frequently occurring side-by-side in the same vessel. The cellular exudate found in the intima appears to arise by a direct migration of the wandering cells from the surface of the artery."

Although inflammatory diseases of the intima have long been recognized, we, nevertheless, feel that the exact character of the lesion is not generally recognized. It was not until recently that there has been specific information concerning this subject, and we would like very much to add supporting evidence to a process already so accurately described by Klotz.

In a previous study upon the reactions of elastic tissue to inflammation we have briefly made mention of the reaction seen in the vessel walls in a case of acute septic meningitis. Following this work we thought it of special interest to study the changes in the arteries of the meninges in the various types of acute meningitis. For this purpose we have selected cases of acute septic, tuberculous, syphilitic, and anthrax meningitis, together with blastomycosis of the meninges and acute anterior poliomyelitis.

The stage of the disease at which the patients died of acute septic meningitis always gave evidence of a simultaneous involvement of the meningeal arteries. At the beginning the intima was found very edematous and the cells widely separated, with a considerable pinkish staining granular debris between them. Whether this can be accurately taken as the initial stage in all cases is difficult to say. However, shortly following upon this edema there was seen a migration of inflammatory cells from the lumen of the vessel into the intimal tissues. Quite often inflammatory cells could be seen passing between the cells of the intimal endothelium, and it appeared as though the endothelial cells were indented or plastic at these points of entrance. The endothelial covering of the intima could be made out in the majority of places and consequently allowed accurate study of the process. With the migration of these cells into the intima the latter became much thicker and more swollen, so that it formed a prominent, puffy inner layer (Fig. 1.) The cells which took part in this reaction consisted for the most part of polynuclear leukocytes, which extended through the entire depth of the intima to the internal elastic lamina. However, associated with the polynuclear leukocytes there were a fair number of lymphocytes and large phagocytic endothelial cells. In some specimens these endothelial cells appeared in great numbers, simulating not a little the grouping of endothelial cells in the more chronic lesions. Furthermore, amid the collections of endothelial cells were seen other cells like fibroblasts arising from the fixed cells of the intima and having elongated oval nuclei and swollen spindle-shaped bodies of clear cytoplasm. These cells appeared in the deep intima, but were

also seen in the intermediate zone of this layer. The endothelial cells were large and round, with pale-staining protoplasm and centrally placed stippled nuclei. These cells were phagocytic for polynuclear leukocytes, lymphocytes, and a dark brown pigment.

In the intima of some of the vessels the pigment in the endothelial cells was not observed, while in others a fair amount of pigment, debris, and cells was found. The endothelial cells were often so crowded with phagocytosed cells and material that it was difficult to make out the nucleus proper. In some instances a number of red-blood cells were also seen in the lesion of the intima. In most of the vessels studied the internal elastic lamina formed the outer boundary of the inflammatory infiltration of the intima. It would appear that the inner elastic membrane is a very resistant layer and limits the extent of the inflammation for a relatively long time, although ultimately the barrier is overcome.

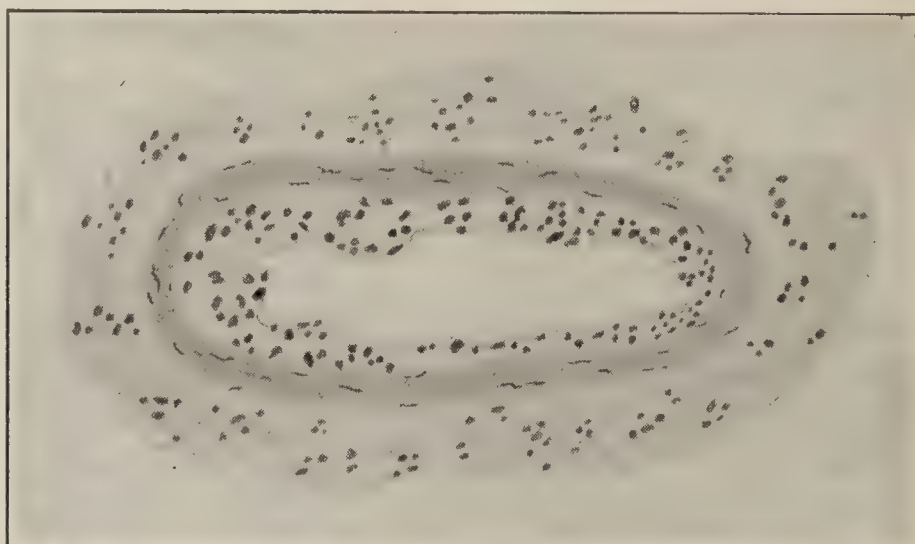


FIG. 1.—Septic meningitis. Acute inflammatory infiltration of intima.

The adventitia is commonly invaded by the exudate which covers the meninges. This exudate is made up of large numbers of polynuclear leukocytes with lymphocytes and a considerable number of endothelial phagocytes. These cells invade the adventitia, but curiously enough stop at the outer border of the media. The striking feature of this is that in the same artery the infiltration of the intima has stopped at the inner elastic membrane, so that the media is left intact. In vessels so affected the media at this time shows no deviation from the normal. It would be well to note that in these vessels there is no demonstrable relationship between the reaction in the adventitia and that in the intima, and hence that the media cannot be the site from which the extension into the intima occurs. Another noteworthy feature is that the adventitia is quite often comparatively free from inflammatory cells while the intima is densely infiltrated, the entire circumference being uniformly affected.

Although the media is uninvolved in the earlier stages, it, never-

theless, becomes involved later on. This takes place by the cells advancing along the lymphatics from the adventitia and to some extent from the intima. The extension from the intima takes place only in a very small part after the inner elastic membrane has been injured by the inflammatory reaction in the intima. The weakening of the elastic layer is demonstrated by a Weigert elastic stain, which shows splitting and granulation of the fibers with inflammatory cells lying between the elastic threads. The fine split threads of elastic tissue bend into the intima, and in many places they can be seen lying among the inflammatory cells close to the parent layer. Notching and granulation of the inner border of the elastic membrane was a very prominent feature in these sections.

When (Fig. 2) the inflammatory reaction involves the media it is more intense in the outer layers of this coat. From this appearance it would seem that the inflammatory reaction in the media



FIG. 2.—Septic meningitis. Splitting of elastic lamina through inflammatory process.

comes for the most part by the extension from the adventitia and advances to the outer border of the inner elastic membrane but rarely goes further. When an inflammatory infiltration was observed in the media the invading cells were sparsely scattered and rarely included all of the types seen in the intima.

The intima of the arteries of the blastomycotic and anthrax meningitis showed extensive infiltration by inflammatory cells, polymorphonuclear leukocytes, together with a fair number of large swollen endothelial cells being the principal types of the exudate. The reaction in the intima was particularly acute. An occasional leukocyte with acid-staining granules was seen. Although the reaction was most marked in the intima, there were a considerable number of inflammatory cells in the media and adventitia. These cells were confined to the coat in which they were found, and the internal elastic lamina seemed to act as a limiting membrane. We found it took a fair time for the inflammatory products to bring

about a sufficient change of the elastic layer to permit diffuse cellular migration, in which the intimal and medial reactions commingle and appear as if they had been part of a single process advancing from without inward. The change noted in these vessels conformed to the more advanced reaction observed in the arteries of acute septic meningitis.

In the arteries of tuberculous meningitis the vessel wall was only mildly involved, compared to the types previously described. The intima was moderately swollen, with the presence of occasional lymphocytes and polymorphonuclear leukocytes. The involvement never reached the proportions seen in the more acute infections. Inflammatory cells were seen scattered in the muscular wall, but the reaction was more particularly perivascular in character. This periarterial inflammation with lymphocytes was also the type observed in the syphilitic meningitis and anterior poliomyelitis.

Sections of the acute septic meningitis stained with Sudan III, and hematoxylin showed the presence of fat in the cells contained within the intima. This fat was found in the form of fine granules, some of which were almost globular. It was present in the leukocytes and endothelial cells of the inflammatory infiltration. At times a slight fatty change was noted in the deep intimal tissue bordering on the elastic layer. The fat here appeared smooth and homogeneous. This was only slight in amount, and was not the rule, most of the fat being intracellular.

The presence of large endothelial cells in the intima of arteries have been described by Klotz and Anitschkow. Anitschkow fed rabbits with cholesterin mixtures and demonstrated doubly refractile lipoid bodies within endothelial cells in the intima of their arteries. Klotz noted that "similar cells may be commonly demonstrated in human arteries where the fatty degeneration occurs in the superficial layers of the intima, and when such specimens are cut (frozen) on the flat they are seen as compact aggregations in which the lipoid substance is almost entirely intracellular." As previously mentioned we found endothelial cells in the intima containing fatty granules and small globules as well as other endothelial cells containing phagocytosed cells, fat, and blood pigment. We would draw particular attention to the phagocytic character of the endothelial cells observed in our specimens, and to note that it is not at all unlikely that the endothelial-like cells noted by other observers are of the same type.

It is almost impossible to definitely indicate the final results which occur in the arteries that we have examined, as these particular diseases are so uniformly fatal. However, there are several points to bear in mind which direct our attention toward the probable outcome of the arterial lesion. We have seen that the inflammatory process in the intima is accompanied by progressive as well as degenerative changes. Fibroblasts, several layers deep, were found

proliferating in the swollen intima, and fatty substances were present in the superficial infiltrating cells and only rarely in the region of the internal elastic lamina. Most of the lipoid substances were intracellular, and much of it would, in all probability, have been removed by active metabolism in the stage of healing. In the end the fibroblastic proliferation would have brought about repair of the injured layer, leaving the intima thickened and hyalin in appearance.

Virchow demonstrated that the intima, like other non-vascular structures, may be the seat of inflammation, and that a proliferation of its own cells leads to the nodular masses on the surface. Moreover, the endarteritic thickenings of the aorta about the intercostal arteries have been recently proved by Klotz to be of inflammatory origin. He found the reaction an inflammatory one, accompanied by progressive as well as degenerative changes in the tissues of the intima and repair accomplished by a proliferation of the connective tissues of the inner layer of the intima. It was further noted by him that inflammatory reactions of the intima of longer duration are always accompanied by a connective-tissue disturbance of a proliferative kind, and from them there develop the intimal thickenings of the "hyalin" type.

There is still another process which must not be passed unnoticed. the great tendency for thrombosis in these vessels. With the marked involvement of the intima and at times disorganization of this layer we have a factor enhancing the coagulation of blood. Added to this the narrowing of the vessel lumen, slowing of the blood stream at irregular points, and an abnormal reaction of the inflamed tissue still further assists in producing thrombosis. In many of the vessels of acute septic infection there is total destruction of the intima, with early thrombosis. Fibrin and inflammatory cells are found in the deep intimal tissues and take a position between the split fibers of the internal elastic lamina. The repair of such a process would eventually lead to obliteration of the arterial lumen. This alteration in the structure of the vessel has been variously indicated as "endarteritis obliterans" (Friedländer), "endarteritis productiva" (Orth), "thromboangiitis obliterans" (Buerger), and a type similar to the last described by Winiwarter.

In our discussion we wish to be clearly understood that our observations have been limited entirely to the study of acute cases. Further, although we have studied a variety of affections of the meninges, our opinion as to the ultimate outcome of the changes found is based for the most part upon the reactions occurring in the arteries of septic meningitis during the acute stage. The organisms associated with this affection are the most common factors in the production of acute purulent lesions in the body. They are commonly associated with septicemia, pyemia, and ordinary infections, to which we are all subject. Our findings are,

therefore, very suggestive that if the opportunity is taken to study the systemic vessels in the various stages of infectious diseases, the inflammatory character of the endarteritic process as described by Klotz, could in many instances be determined.

CONCLUSIONS. The intima, although a non-vascular structure, is capable of suffering an acute inflammatory reaction.

This inflammatory infiltration occurs by the direct migration of the wandering cells from the lumen of the vessel.

An inflammatory reaction in the intima is not always accompanied by the presence of inflammatory cells in the remaining coats.

Furthermore, a simultaneous infiltration of the intima and adventitia by inflammatory cells may occur without any involvement of the media.

Accompanying the inflammatory reaction in the intima there are alterations in the elastica interna similar to those described in a previous paper.

Although we have had only the opportunity of observing early proliferative changes in the lesions studied, we, nevertheless, are of the opinion that ultimate repair is brought about by the continued proliferation of the fixed cells of the intima.

I am indebted to Dr. S. R. Haythorn for the specimens of nodular syphilitic meningitis.

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TISSUE REACTIONS IN EXPERIMENTAL HYPERCHOLESTERINEMIA

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(From the Pathological Laboratories, University of Pittsburgh, Pittsburgh, Pa.)

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TISSUE REACTIONS IN EXPERIMENTAL HYPER-
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Experiments performed by feeding cholesterin and foods rich in cholesterin to rabbits are attended by definite changes within the organs of these animals. This work was first undertaken by Ignatowski, who thought the changes in his animals were due to a severe disturbance of their protein metabolism. However, Stuckey found that he could produce changes in the aorta of rabbits by feeding them with egg yolk and brain substance, while he was unable to obtain results in rabbits fed upon pure neutral fats of animal or vegetable origin. Chalатов also investigated the effect of egg yolk and brain when used as food for rabbits and found an accumulation of doubly refractile substances within the liver cells with some evidence of cirrhosis. With substances devoid of cholesterin he found the organs of the animals without change. This author later observed that the feeding of pure cholesterin produced results similar to his previous experiments with egg yolk and brain substance. The results which were obtained with egg yolk led Wesselkin to use pure lecithin in an effort to produce these changes. However, his experiments were attended with negative results. On the other hand, Anitschkow employed pure cholesterin as a food for rabbits and found that fatty plaques were developed in the aorta of his animals.

In experiments similar to the above Wacker and Hueck determined that the cholesterin and cholesterin-ester content of the blood serum was increased. Rothschild, Waltmann and Biach, and Sternberg have obtained similar results.

In an effort to produce fatty plaques in the aorta like those observed by the Russian school we fed rabbits through a

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stomach tube with cholesterin in solution. Some of the rabbits received the cholesterin in olive oil, while others were given a sodium oleate cholesterin emulsion (Klotz). The daily dose of cholesterin varied from .28 to .56 gram. Of ten rabbits treated in this manner five showed the presence of fatty plaques in the aorta, while the organs of the remaining animals presented tissue changes of varying degrees.

The individual organs of animals, fed artificially with cholesterin, are not equally concerned in handling the cholesterin. This material calls forth a reaction dependent in its severity upon the amount administered and the length of time it is given. The changes noted consisted of an alteration in parenchymatous structures along with a response of endothelial tissue. Further, there was a marked accumulation of cholesterin and its compounds in some of the organs.

The organ which appears to be more concerned with cholesterin metabolism than any other is the adrenal. Shortly after the development of a hypercholesterinemia, the cells of the gland showed the beginning of an accumulation of cholesterin. This continued until after a period of one hundred and sixty-six days, as in one of our rabbits the organ reached a very large size, being two centimeters in diameter. The cortex of the gland became very much widened while the medulla was little affected. In all of the animals the adrenals presented various degrees of enlargement depending upon the amount of cholesterin they had received. The cells of the zona fasciculata and adjacent cells of the zona reticularis were particularly affected. The enlargement of the cortex depended upon the increase in size of its individual cells. They gradually increased in size until they became large and watery looking. Their protoplasm was either formed of a delicate reticulum or was fairly granular. The character of the protoplasm was a very interesting feature in the sections as the cells which had a reticulated protoplasm contained large numbers of cholesterin crystals and small and large-sized globules. Some of the cells showed fusion of the globules and were occupied by a single large vacuole. Again the cells which had a

granular protoplasm contained only small amounts of cholesterol crystals. It appeared as though the cells with the reticulated protoplasm were more intimately concerned with the storing up of the cholesterol materials. In sections stained with Sudan the cells were found to be loaded with an orange-colored fat. The Nile blue reagent showed most of this fat blue, although red-tinged globules were also present. The cells of the zona glomerulosa were filled with fat in the more advanced cases. The important part which the adrenal undertakes in endeavoring to store the cholesterol compounds circulating in the blood was demonstrated by the amount of anisotropic lipid in the adrenals of these animals. Ordinarily the gland contains relatively little anisotropic lipid when compared to the enormous amount of doubly refractile substances seen in the adrenals of a treated animal. Landau has pointed out that in rabbits fed with cholesterol, the adrenal receives the cholesterol in a combined form from the blood and endeavors by a process of storage to relieve the body of the excessive amount of cholesterol in the blood. Sternberg noted that there was no hyperplasia of the cortical cells but rather an overloading of the individual cells with cholesterol materials. From what we have observed in our sections we are inclined to believe that the adrenal is used as a depot for cholesterol compounds where it can be stored to relieve the blood of an excessive load.

Another organ which bears a very close relation to cholesterol metabolism is the liver. Not only are the liver cells themselves intimately concerned with the storage of the cholesterol compounds, but the lining cells of the sinusoids also contain lipoids and very often show proliferation. In the liver of a rabbit treated with seventy-five grams of cholesterol in nineteen days, the Kupffer cells stood out very prominently. They contained a considerable amount of lipid material and were often loaded with fat globules while the liver cells were free. The part played by the Kupffer cells in the liver of this rabbit was so prominent that the fatty material had the appearance of being arranged in rows

which were limited to the sinusoids. The liver cells first contained fat in that part of the cell bordering the sinusoid and then later became filled with fat. It looked as though the liver cell obtained the fat from the Kupffer cell after the latter had taken it up from the blood. This relation of Kupffer cell to liver cell was first noted by Anitschkow and later studied by Rothschild. The fatty substances within the liver cells were mostly found within the inner half of the lobule. In this region the liver cells stored large quantities of fat so that the liver cells became vacuolated and often presented evidences of degeneration. The liver of this animal contained many more doubly refractile bodies than the adrenal. In animals which had been fed large amounts of cholesterin over a long period of time, the fatty accumulation within the cells gradually occupied more of the lobule. With polarized light the cells showed the doubly refractile globules in abundance, while many crystals and true plates of cholesterin were also noted. With maintenance of a hypercholesterinemia over a long period of time the liver accumulates an enormous amount of cholesterin compounds in an endeavor to furthermore relieve the body of the excess of cholesterin materials in the blood. Rothschild has found that the liver secreted an excess of cholesterin in the bile when a sufficient grade of hypercholesterinemia was established. A morphological and chemical increase of cholesterin in the liver has been noted by Anitschkow and Chalатов and also by Weltmann and Biach.

The function of storing cholesterin is also undertaken by the spleen. This organ does not present the uniform evidence of a response of its tissue as in the case of the adrenal and the liver. Although this is broadly true, we found the organ responded very rapidly when the cholesterin feeding was forced. A rabbit fed with seventy-five grams of cholesterin in nineteen days showed a most unusual picture in its spleen. The organ was twice its normal size, quite firm and of a lighter color than usual. Paraffin sections of the organ showed the sinuses filled with large cells. Many of them

were large and foam-like, while others were definitely phagocytic and contained many acid and basic tinged granules along with a brown pigment which contained iron (Nishimura). Eosinophiles were frequently observed. This reaction was a part of the proliferative reaction of the lining cells of the sinuses which in some was so advanced that they were almost closed. This change was suggestive of the alteration seen in Gaucher's spleen. In Sudan sections the organ was loaded with orange stained fatty substances within the large cells filling the sinuses. A small amount of this fat was anisotropic. The reaction which occurred in the spleen of this animal was greater than that noted in any of the other rabbits. However, in some of the animals treated a longer time the spleen contained more anisotropic lipoid. The alterations noted in all of the spleens were limited to the arterial sinuses. Only occasionally were the arteries of the spleen affected and then the change consisted in a fatty degeneration of the media. The intima was not affected.

A structure which was actively concerned in the using up of the cholesterin was the corpus luteum. Two of our rabbits were pregnant during the feeding experiments. One of these animals received 76.72 grams of cholesterin in olive oil in one hundred and sixty-six days, while the other was fed 61.6 grams of cholesterin in a sodium oleate emulsion over a period of one hundred and fifteen days. Their ovaries were almost entirely occupied by corpora luteal tissue, only a small rim of ovarian structure containing Graafian follicles remained. As in the adrenal two types of cells were noted. The great majority of cells were large and had a granular reticulated protoplasm; other cells were seen with a delicately vacuolated meshwork in their protoplasm. In the latter many cholesterin clefts were observed, while the cells of the former type rarely contained them. The cells which contained cholesterin clefts were of the foam type and appeared to be more actively engaged in caring for cholesterin materials. They had no relationship with the arteries. They appeared to be a more healthy lutein cell. With fat stains the lutein cells were seen to be crowded with fatty substances.

Most of the fat was in the form of small globules. With Nile blue the majority of it was colored blue. The tissue was loaded with doubly refractile bodies, crystals and needles of cholesterin.

Aside from the reactions found in the structure of certain organs, there was another manifestation of increased activity on the part of the body in combating the excess of cholesterin compounds in the blood. The intima of the aorta and pulmonary artery, as well as the endothelium of some of the smaller arteries and arterioles, had responded so that definite alterations could be made out in their structure. Of these structures the intima of the aorta presented the most pronounced change. The ten animals in our series received an amount of cholesterin varying from 8.12 grams to 76.72 grams over a period of from nineteen to one hundred and sixty-six days for the individual rabbit. Five of the ten rabbits presented fully developed fatty plaques in their aortas. There was a proliferation of several layers of the large foam cells in the intima which was accompanied by fibroblastic increase with splitting of the internal elastic layer and the development of new elastic threads. The large foam cells were quite frequently found interposed by proliferation between the muscle cells of the inner half of the media. In Sudan stained sections the cells were filled with globular lipoid, most of which in Nile blue preparations was tinged blue. Many crystals and needles of cholesterin were seen. With polarized light the foam cells were observed to contain large numbers of doubly refractile bodies. There were many cholesterin clefts in the deeper intimal tissues. Klotz and myself have undertaken a more intimate discussion of the intimal changes of the aorta in another paper.

A very interesting condition was found in the pulmonary artery in two of our rabbits. One of these animals receiving 76.72 grams of cholesterin in olive oil during one hundred and sixty-six days showed fatty plaques extending into the larger branches of the pulmonary artery in the lung. The other rabbit was fed 61.6 grams of cholesterin in a sodium oleate cholesterin emulsion over a period of one

hundred and fifteen days and presented well developed fatty intimal change in the small ramifications of the pulmonary artery. On section of the lungs of this latter animal these areas were seen as milk-white dots scattered over the cut surface. Sections of these areas proved to be an intimal proliferation of the small arterioles, consisting of large foam cells like those observed in the fatty plaques of the aorta. The structure of these areas was identical with those seen in the aorta. Some of the plaques had developed to such a degree that the lumen of the vessel was almost occluded. Frequently several plaques growing from different points in the intima were noted in a vessel. In some of the arteries there was an advanced splitting of the internal elastic layer with the foam cells between the split fibers. The foam cells were also found placed between the muscle cells of the media. These areas of proliferation showed the individual cells to be loaded with doubly refractile bodies along with many crystals and needles of cholesterin. Well formed fatty plaques were found in the carotids, iliacs, and femorals. In a similar manner varying degrees of intimal proliferation were noted in the branches of the coronary and renal arteries. In the arteriolæ rectæ of the kidney there was a proliferation of cells similar to those described in the larger arteries. The lining cells of these small vessels grew to such an extent that they could be recognized in the gross as white radiating streaks in the pyramids and microscopically were seen to form large islands of cells in the interstitial tissue. They were engorged with doubly refractive lipoid and actively engaged in cholesterin storage. Again where proliferation had not occurred, the lining cells of small capillaries often contained doubly refractive lipoid globules. This was particularly noted in the lining cells of the capillaries of the heart muscle of a rabbit which had received forced cholesterin feeding.

The association of a number of organs with cholesterin metabolism has been rather widely investigated. This is more particularly true of the adrenal and liver. More stress

has been placed upon the part the adrenal has to do with cholesterin metabolism than any other organ. As early as 1882 Gottschau pointed out that in pregnancy there is an hypertrophy of the cortex of the gland. Albrecht and Weltmann, Hueck and also Landau have emphasized the increase of cholesterin and fat in the blood during pregnancy. Sternberg found that the essential feature of the enlargement of the cortex in pregnancy and cholesterin feeding was a storage of fat and lipoids. This author further indicated that the condition of hypertrophy depended upon a hypercholesterinemia and only indicated a storing function of the adrenal cells. That the adrenal acts as a depot for cholesterin has also been strongly supported by Landau, Hueck, and Rothschild. The last-named author in his work on extirpation of the adrenal found that the vital significance of the organ depended upon the fact that it was able to store cholesterin. Stewart was able to remove both adrenals in gravid animals without affecting them and noted that the life of these animals could be preserved a long time by injecting cholesterin into the blood. Herxheimer has cited the case of an animal lacking both adrenals, but having a small accessory adrenal which lived a long time under cholesterin feeding and presented marked alteration of its aorta. By starving cats Gardner and Lauder found that the cholesterin of the adrenal diminished during the starvation period while that of the blood still remained constant. In fasting animals Rothschild was of the opinion that the cholesterin increase in the vital organs depended upon increased cholesterin in the blood and this in turn upon an accentuated destruction of fat tissue. He further compared the results of his supra-renalctomy experiments with what he observed in fasting animals and found that there was a hypercholesterinemia and hypercholesteastosis in both instances. Along with these conditions there was a loss of body weight with a melting down of fatty tissue. With the development of a hypercholesterinemia in these experiments Rothschild also found that the liver played a very important part in protecting the

body against too great an accumulation of cholesterin in the blood by secreting an increased amount of cholesterin in the bile. Weltmann and Biach have demonstrated that carnivorous animals are more protected than herbivorous animals on account of the uniform secretion of cholesterin in the bile independent of conditions producing a hypercholesterinemia. However, Weltmann and Biach have also pointed out that herbivorous animals cannot excrete all of the cholesterin administered and it is consequently stored in their various organs. These authors have found an increase of cholesterin compounds in the liver cells, while Anitschkow and Chalatow have also noted a morphological and chemical excess of this material in the liver in feeding experiments.

Concerning the changes noted in other organs we find that Anitschkow and Saltykow have described the fatty plaques which occurred in the aorta of cholesterin-fed rabbits. In connection with suprarenectomy experiments Rothschild described the active part played by the Kupffer cells of the liver in dealing with cholesterin materials. According to Aschoff and Landau, the endothelial tissue of the spleen, lymph nodes, and bone marrow, together with the adrenal and Kupffer cells of the liver, constitute a very important intermediary apparatus in cholesterin metabolism. Anitschkow has called attention to the proliferation of large cells in the spleen and in his feeding experiments has described the accumulation of cholesterin compounds in the intima of aorta, spleen, and lymph nodes.

From a study of our findings we were particularly impressed with the fact that the organs of our animals contained large amounts of cholesterin free or combined. The adrenal and liver were very active in this respect and had accumulated so much of this material that the cells frequently presented signs of being taxed beyond their capacity. In the liver the Kupffer cells became filled with these materials very early. As the process of feeding advanced the amount of cholesterin in these organs always increased. In forced cholesterin

feeding the endothelium of the small capillaries and the endothelium of the arterial sinuses of the spleen respond in a marked degree along with the adrenals and liver. As the feeding is continued over a longer time other endothelial tissues respond, as that of the arteriolæ rectæ of kidney, intima of aorta and its branches as well as the pulmonary artery and its finer ramifications. Thus we were impressed by the response on the part of the body of some of its individual tissues. As each succeeding organ is called upon and becomes inadequate to care for the cholesterin load, a time is reached when the equipment of the particular tissue is inadequate to satisfy the demand. Proliferation now occurs in the various organs as well as in the lining cells of the arteries and small capillaries.

As the amount of cholesterin gradually increases within the body, the demand for greater assistance in caring for this material becomes imperative. The liver and adrenals comprise the most important organs first called on. When the work becomes too heavy for them, they are assisted by the corpora lutea, spleen, and endothelium of the blood vessels. Thus we are of the opinion that the alterations observed in hypercholesterinemia constitute a sequence of compensatory acts on the part of the body in an attempt to rid the blood of an excess amount of cholesterin which cannot be properly excreted.

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GLIOMA OF THE CEREBELLUM WITH METASTASES

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(From the Pathological Laboratories, University of Pittsburgh, Pittsburgh, Pa.)

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GLIOMA OF THE CEREBELLUM WITH METASTASES.*

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Glioma or glioblastoma has been defined as "a tumor of epiblastic origin in which the cells tend to differentiate into neuroglia cells." It has been designated by some of the older writers and also in fairly recent literature, gliosarcoma. Most writers who offer this term do so without any explanation for its use, but Adami states that tumors of this type may be called gliosarcomata only in a histological sense, to indicate that they are hylic tumors of the vegetative type, but it must always be remembered that they are derived from epiblast and not mesoblast. It is probable that the name gliosarcoma was applied to this type of tumor on account of its resemblance to sarcoma in many of its gross characters. There is one great difference, however, in the manner of growth, which is in the want of invasion of blood vessels and the formation of metastases. The rate of growth, nature of cells and character of the stroma of different examples of glioma differ so widely that, although because of its position it is nearly always clinically malignant, it oftentimes appears histologically as a benign tumor. Thus when we consider sarcoma as a definitely malignant mesoblastic tumor the term gliosarcoma is entirely a misnomer.

Some interesting points have been described by Weigert and later by Mallory in connection with the ependyma and its associated gliomata. Ependymal cells contain small oval and rod-shaped granules which are stained blue by phosphotungstic acid hematoxylin stain. They appear in small clusters, varying in number up to thirty, at some distance from the nucleus of the ependymal cells as well as in smaller numbers in certain glioma cells. Their significance

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is not known, but they are characteristic of ependymal cells and are sufficiently characteristic to recognize ependymal tumors. Ependymal tumors also form small gland-like structures and in some cases fair-sized cysts lined by quite normal looking ependymal cells.

Some of these features and the question of the distribution of metastases are interesting points to be considered in the following case:

C. K., aged 28 years, was admitted to the Mercy Hospital January 15, 1915, under the service of Dr. H. H. Donaldson and giving a history of repeated attacks of headache over a period of two years. These attacks were at first slight, but gradually grew more severe and of longer duration until the patient at the time of admission was suffering with a very severe, almost continuous headache. About one year ago he noticed dimness of vision, which also progressed until about three months ago he became totally blind. There was no history of projectile vomiting. Just before admission to the hospital the patient developed complete paralysis of both lower limbs.

The pupils were dilated and did not react to light. Accommodation of eye grounds showed complete atrophy of both optic nerves. Knee reflexes were slightly increased. A decompression operation was done through the right parietal region. An intradural abscess developed at the site of operation and the wound did not heal. Patient died March 17, 1915.

AUTOPSY (eight hours after death by Drs. Maclachlan and Jacob). — The body was that of a well developed and well nourished adult male. There was a moderate rigor mortis and post-mortem lividity present on the dependent parts. The scalp showed a large semicircular incision over the right parietal region, the ends of which were about an equal distance, 3 centimeters from the tip of the ear. The wound was only partially healed and from its upper border a yellowish green purulent fluid could be expressed. The pupils were dilated and equal. The chest was well formed; the abdomen was flat and the genitalia and extremities quite normal.

HEAD: The skin over the parietal region about the incision showed a little thickening. The incision was only partially healed and could be easily broken open. Its upper border was bathed in a yellowish green fluid. A trephine wound about 2 centimeters in diameter was seen about the middle of the parietal bone. At the bottom there was a moist reddish tissue having the gross appearance of granulation tissue.

The skull cap was of normal thickness. The dura over the parietal region was much altered. It was slightly adherent to the margin of the wound but could be easily separated. Over an area measuring 5 x 4 centimeters the dura was much thickened, edematous, of a glassy red color

and in the center showed a round greenish yellow patch which corresponded to the trephine opening. The area was in direct connection with an abscess lying below the dura at this point. The abscess was flattened and localized. It measured 4.5 x 4 centimeters and in its deepest portion was almost 1 centimeter in thickness. The abscess was perfectly encapsulated and about it the dura was fixed to the brain. The edematous thickening of the dura extended for a little distance beyond the borders of the abscess. The abscess was filled with a yellowish green purulent fluid. The brain substance was not involved by this purulent process save by slight compression. The pia mater under the abscess showed edema and thickening, but the pia elsewhere showed no change. No tumor masses could be recognized by the naked eye in the meninges.

BRAIN: Weight, 1,600 grams. The brain itself appeared to be under tension. It was a soft wet brain. The convolutions generally were a little flattened but one could not distinguish any difference in texture by palpation. On examining the base of the brain a mass measuring about 2 x 3 x 1 centimeters was found which occupied and infiltrated the optic chiasm and extended backward to and involved the corpora mammillaria. The mass was a soft, friable grayish red, very vascular, almost gelatinous tumor. Its outlines were very irregular and it was firmly adherent to the under surface of the brain.

When the cerebrum was sectioned the tumor mass was found to have infiltrated and practically destroyed the optic chiasm and its tracts, the tuber cinerium and the corpora mammillaria. It was in direct continuation of a mass which completely filled the third ventricle. The cerebral hemispheres were moist and slightly congested. Further examination showed both lateral ventricles slightly enlarged. The lining of the ventricles contained a number of small papillomatous masses. These masses were somewhat spherical in shape and varied in size from very minute granules to masses about .5 centimeter in diameter. They were rough and granular, looking not unlike warts. They were soft and friable and on section appeared to lie entirely in the ependyma. The rest of the ependyma was smooth and normal. The choroid plexuses were clear. The basal ganglia were edematous and glassy. Section of the crura cerebri showed both of them to contain in their central portions soft grayish masses. These masses were small, soft, and gelatinous. The outlines of these masses were regular, but indistinct, and they joined almost imperceptibly with the surrounding tissues.

The cerebellum felt soft and was somewhat more rounded than usual. On section through the cerebellum a large soft gelatinous tumor was noted in its central portion extending from the roof of the fourth ventricle and progressing laterally to involve most of the white matter of the cerebellum. The tumor was oval in shape and its cut surface measured 5.5 x 4.5 centimeters. It was soft and friable and in color varied from a dull gray to a reddish gray. The tumor had invaded and obliterated the third and fourth ventricles. Only the upper part of the cord was removed. Section of the cord showed the central canal to be entirely obliterated.

It was filled by a glassy dull grayish material exactly similar to the tissue of the tumor in the cerebellum. In the medulla this condition was more marked and the tumor had a wider distribution about the circumference of its cavity.

The autopsy of the remaining portion of the body showed nothing of particular note. There were some old fibrous adhesions in both pleural cavities. The lower lobe of the right lung showed hypostatic pneumonia. The heart was negative. The intima of the aorta showed a little fatty change. There were no abnormalities in any of the abdominal viscera.

DIAGNOSIS. — Glioma of cerebellum; extension of glioma into third and fourth ventricles and about central canal of cord; internal hydrocephalus (slight); edema of brain; compression of convolutions; optic atrophy; intradural abscess; chronic leptomeningitis (diffuse); old bilateral pleural adhesions; hypostatic pneumonia (right); fatty changes in aortic intima.

Microscopical. — The main tumor was quite dense and very cellular. The cells varied somewhat in size and contained varying amounts of protoplasm. The cells were usually either round or oval, but some of them were flattened and spindle shaped. The nuclei were round or oval in shape and of good size. The nuclei as a rule stained well, but only a few cells showed nucleoli. Most of the section was made up of masses of cells showing no particular arrangement. Cells were found in columns and in masses. A few areas showed groups of cells which had formed gland structures. These structures were few in number and none of them were well formed. Around some of the blood vessels, rosette formations, such as are seen in retinal gliomata, were found. There were many masses of cells resembling giant cells, but true tumor giant cells were rare. The small round and rod-shaped protoplasmic granules, which are found in ependymal cells (Mallory, Wright), were seen in many of the cells. Some of the cells contained clusters of these granules resembling nuclear material in the center of a large giant cell. Neuroglia fibrils, both coarse and fine, were found in large numbers. Fibrous tissue stroma was found about the blood vessels and was small in amount. Several small hyaline areas suggesting old hemorrhages were noted in the mass. The blood vessels varied in number and size in different parts of the mass. Many of

the blood vessels were thin walled, but others had fairly thick walls showing hyaline degeneration of the media and adventitia.

Sections of the outer cerebellar convolutions showed some tumor tissue located between the convolutions and not directly continuous with the main tumor. This tumor tissue was scattered diffusely and did not form distinct nodules. Many convolutions in the section showed small masses of tumor tissue. These small masses had destroyed the pia mater and had infiltrated the outer layers of the white matter to a considerable extent. The structure of these masses was somewhat more fibrous (pia) than the central mass. The blood vessels were more numerous and the rosette formation around them was more marked. In all other respects these masses were similar to the large mass in the central portion of the cerebellum. The tumor masses found in the pia mater were not nodular masses such as those found in the ventricle but were thin and diffuse, covering a very large area on the surface of the brain and cord.

Sections of several masses from the lateral ventricle showed these tumors to have a common histological structure, the differences being mainly in size and manner of growth in the different locations. The masses which developed upon the ventricular surface of the optic thalamus formed small papillomatous projections. They lay quite superficial and were composed of a dense cellular tissue in which no trace of the normal ependyma could be found. On either side of these masses the ependyma was normal and to all appearances unaffected. At the angle of the ventricle was a mass of peculiar appearance. It had evidently developed upon one surface and had grown to and invaded the opposite surface. Where the tumor had been implanted the ependyma was completely lost, but between several small masses, closely arranged, the ependyma remained. The appearance of these masses was characteristic of glioma. The cells were irregular in size and shape and were epithelial in type. There was no definite arrangement of cells in any part of the tumors. Mitotic figures were not very numerous.

Neuroglia fibrils were noted in all parts of the masses. The tumor had invaded the underlying brain tissue to a slight extent. Blood vessels and fibrous tissue stroma were present. The ependymal cells near the tumors showed very well the characteristic protoplasmic granules and these granules were also noted in some of the tumor cells. The brain beneath the tumor was edematous. In a clear zone around some of the vessels lymphocytes were found.

Sections were taken from several points of the cerebral surface. The brain tissue itself was for the most part without change. In many sections of the cerebrum one or more small nodules of gliomatous tissue were located in the pia mater. These nodules were composed of cells similar to the other tumor masses examined. They contained more fibrous tissue, particularly in the boundaries. They seemed to have grown in the meshes of the pia arachnoid, which accounts for the greater amount of fibrous tissue than was present in any of the other tumors. These nodules did not in all cases involve the brain tissue, and the few which did infiltrated only superficially. A peculiar condition was noted in some of the masses in that they had a very definite covering of endothelial cells with a fairly large amount of fibrous tissue beneath, giving them an encapsulated appearance. The cells in these masses showed more definitely the protoplasmic granulations than any of the other tumors.

Sections of the tumor mass about the central canal showed a denser and less cellular appearance than any of the other masses. The cells were similar and of various sizes, round or oval cells were found as in the tumors of the cerebellum. The nuclei stained well and only a few of them showed nucleoli. The cells showed a rather curious arrangement. They were grouped in numerous small masses and showed a tendency to form columns. Some of these columns were crescentic in outline while others had formed small glandular groups of cells. There was always some stroma in the lumina of these gland-like structures. Aside from these, many masses having no definite arrangement were found. Around some of the blood vessels the "rosette" formations

were evident. Nuclear figures were not numerous. The stroma was abundant and evenly distributed. There was no evidence of encapsulation of the mass and at the edge of the tumor the stroma of the surrounding cord seemed to extend in and from the stroma of the tumor. The boundary of the tumor could be distinguished only by the absence of tumor cells. It was fairly sharply demarcated. There was no normal ependyma to be seen anywhere in the canal. The cord around the tumor seemed a trifle more dense than normal, but this condition was not marked on account of the blending of the tumor stroma with that of the cord. The blood vessels were not numerous. All the vessels were of medium size and had fairly thick walls which were composed chiefly of fibrous tissue. Some of the blood vessels showed hyaline degeneration of the media and adventitia. Throughout the stroma many fine and also a few coarse neuroglia fibrils were noted. Most of the fibrils were very twisted and tortuous, only a few straight ones being seen. Some of the cells showed the protoplasmic granules characteristic of ependymal cells. In the pia arachnoid around the cord, particularly in the anterior sulcus, a small but diffuse growth of gliomatous tissue was noted. It was located entirely within the meninges and had no connection with the cord at any point.

Tissues from various parts of the lung and other distant organs were examined, but none of them showed any evidence of metastases.

Briefly reviewing the principal points of the case, we have a young adult giving a typical history of brain tumor extending over a period of two years. The autopsy showed a large glioma of the cerebellum which had extended into and obliterated the fourth ventricle and occupied most of the central white matter of the cerebellum. Smaller masses of a similar character were distributed upon the ependyma of the lateral ventricle, the central canal of spinal cord and the leptomeninges. All of these masses were very cellular,

unencapsulated, infiltrating and, to all microscopical appearance, malignant in character. Gland-like and rosette structures were noted in many parts of the tumors. The masses in the ventricle were discrete and had a nodular papillomatous structure, but the pia mater of the cerebrum, cerebellum, and cord contained a patchy growth of gliomatous tissue extending over a considerable area, involving much of the surface of the brain and cord. No masses were found in any organs outside the cranial and spinal cavities.

In the literature the general opinion is expressed that gliomata of the brain do not metastasize in the ordinary way and secondary growths outside the central nervous system are virtually unknown. Aschoff discusses a number of cases of brain tumor, and after a thorough review of the literature concludes that metastases of cerebral glioma are very rare. He quotes one case by Moeller in which metastases were found in the lung, intestine, and kidney, but as the diagnosis in this case was doubtful the alleged secondaries cannot be accepted as gliomata. Pels-Leusden presents a case of glioma of the fourth ventricle which showed metastases in the cord and leptomeninges but did not involve any other structures. Stroebe reports a number of cases beginning in various parts of the brain, particularly in different portions of the ventricles. All of these cases showed metastases in other parts of the brain and cord, but none of them showed any evidence of regional metastases. Mallory reports three cases, one arising in the tissue over the sacrum and two others in the fourth ventricle. No metastases were found in any of these three cases. He says that gliomata, although infiltrating and malignant in character, never invade blood vessels and thus do not metastasize to distant organs. He, however, speaks of one case of glioma of the sacrum, in a woman forty years of age, in which the tumor was excised, recurred and later involved both groups of inguinal glands. The tumor in this case was of long duration and is a most unusual one.

Schmenke reported a case which in many features was

similar to the present one. There were present a large infiltrating glioma in the cerebellum and smaller masses of a similar nature in the ventricles, central canal of spinal cord, leptomeninges and also in the nerve trunks of the cauda equina. No mention of metastases in any other organ is to be found in the report of this case. Adami distinguishes between two varieties of glioma of brain; a hard type which is usually found in the ventricles and soft variety which may appear elsewhere in the brain. He says that neither of these types show any tendency to metastasize to distant organs.

Older classifications recognized two types of glioma, one the simple glioma which was benign in character and the other, the gliosarcoma, so called because of its clinically malignant nature. This classification is at best rather arbitrary while the use of the term gliosarcoma is histologically not correct. Robertson gives a classification of all tumors of nervous tissue origin in which he includes glioma. The tumors are classified according to structural malignancy, but as in some cases the differences are not marked it is difficult to place them in any particular class. Furthermore, gliomata are a definite class of tumors and are to be differentiated from tumors of nerve cells.

The classification of gliomata according to their seat of origin is fairly satisfactory. In this we have retinal gliomata, ependymal gliomata, and gliomata of the stroma of the brain and cord. There are a number of marked differences in each of these classes distinguishing them from the other two. Retinal gliomata are rapidly growing, metastasizing malignant tumors. They form very definite "rosette" masses around blood vessels. Ependymal tumors differ among themselves within a certain range. They vary greatly in size, rate of growth, stroma, formation of fibrils, power of infiltration and degree of encapsulation, but none of them metastasize outside of the brain and all are only locally malignant. Weigert and Mallory have described glands and cystic structures, lined by ependyma, which are characteristic of tumors of ependymal origin. They have also described

a type of protoplasmic granule which appears characteristic of this class of tumor. The third class is a type which resembles the second in malignancy. It varies from a tumor having a fairly well developed capsule to one which is definitely infiltrating. Tumors of this class showed a solid structure which contains no glands, cysts or rosette formation and does not show the cellular granules previously mentioned. These glands, cysts, and granules, which have been mentioned as characteristic of one or the other type of glioma, may at times be found in one of the other classes, but never with such uniformity and in such numbers as in the tumors of which they are characteristic. Thus even though these tumors are composed of the same embryonal type of cell it is possible to differentiate several distinct and separate classes.

If gliomata do not metastasize by the blood and lymph channels what reasons can be given for multiple tumors in the brain and cord such as were found in this case? Some tumor masses, no doubt, are the result of spontaneous gliosis of parts of the brain other than the site of the original tumor. This can hardly be the reason for multiple growths in this instance, for the tumors of the ventricle appeared as small nodular papillomatous, very cellular, infiltrating masses and resembled true tumor more than gliosis, while masses of gliomatous tissue were found in the pia arachnoid mater entirely free from the brain. A condition of primary gliosis could not arise in this location. The fact that the leptomeninges were involved would also rule out the theory that these tumors were derived from multiple primary foci in the glial tissue. The only means of production of the multiple tumors lies in metastasis from the cerebellar mass by way of the cerebrospinal fluid. It appears very probable that cells from the larger, original growth in the cerebellum and fourth ventricle were carried by the cerebral fluid through the aqueduct of Sylvius or the foramen of Magendi to the locations at which the other masses were found. The fact that no metastases were found in the lungs or other organs indicates that the cerebral fluid

alone was capable of transferring the tumor cells to suitable areas for implantation.

It is an interesting fact that tumors of this nature showing no capsule and infiltrating the surrounding tissue to a marked degree do not invade blood vessels or lymph channels and do not form metastases in different organs. Gliomata of the retina do metastasize and there is one reported glioma of the tissues over the sacrum which after long duration involved both groups of inguinal glands. Both of these types originate outside the central nervous system or at least outside the cranial and spinal cavities. The cells from which these tumors arose were primarily adapted to conditions widely different from those of homologous tissues in the central nervous system. These extracranial tissues are capable of growing under conditions and in locations not available for the cerebral glia. Metastases of cerebral gliomata are mainly found in the tissues of the central nervous system or rarely in the investing membranes. Glia cells are highly specialized and it would appear that a reversion to embryonic qualities by the cells of these tumors is not sufficiently complete to endow these cells with marked vegetative qualities permitting them to grow in any environment but their own. For this reason, even if glial tissues could and did infiltrate blood and lymph vessels, they would probably not form secondary growths in remote organs.

The protoplasmic granules found in the cells of this tissue are interesting. They have been proved to be definite intracellular structures and are not the ends of fibrils nor have they any connection with neuroglia fibrils. Their significance is not known but they must bear some importance, for their occurrence is very uniform in the normal ependymal cells and quite frequent in tumor cells.

CONCLUSIONS.

Although gliomata of the brain do not invade blood and lymph channels or form metastases in distant organs, they do form metastases in the brain and cord by means of cerebrospinal fluid.

The reason for this may be found in the fact that glia cells are highly specialized and cannot grow when removed from their natural surroundings.

Even though gliomata of brain do not metastasize to other organs many of them should be considered histologically malignant or at least locally malignant on account of their power of infiltration, rapid rate of growth and embryonal character of the cells.

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CHONDROMA OF THE PELVIS¹

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NUMEROUS articles have been written upon true chondroma and other forms of cartilaginous tumors arising from various parts of the body.

These tumors whether enchondroma, ecchondroma, osteochondroma, chondrosarcoma, or osteochondrosarcoma were, by most authors and especially those of the earlier times, classified as enchondroma. Since 1836, when Jean Muller gave the first complete description of enchondroma, until the present day, a hundred or more articles upon the subject are readily found in the literature. Most of these articles appeared in the French and German literature before 1890. Since then relatively few reports have appeared and only a few of them, dealing mostly with the report of cases, in the English. In this paper, I have attempted to summarize the work of others as well as give a description of three cases of enchondroma of the pelvis coming under our observation.

Chondromata are tumors composed of cartilage-cells with a varying amount of matrix and connective-tissue stroma. These tumors may be composed of any of the three types of cartilage, hyaline, elastic, or reticular, or may be composed of any two or all three types with variable proportions of each. How-

¹From the Pathological Laboratories, University of Pittsburgh.

ever, the tumor most frequently met with is that of the hyaline variety. In the latter there is an attempt to reproduce the true hyaline cartilage, with relatively few cells scattered sparsely throughout a homogeneous matrix.

Classifying chondromata according to their manner of origin, they are spoken of as ecchondromata or enchondromata. Ecchondromata are outgrowths of cartilage occurring in regions where cartilage is normally present, as in the region of a joint, the larynx or trachea. Such a tumor results through a process of hyperplasia or overgrowth of cartilage. Ecchondromata are homoplastic. The enchondromata on the other hand are masses of cartilage or cartilaginous tumors having no connection with areas of normal cartilage formation. These are considered under the heteroplastic group and develop where cartilage is not normally found.

In the gross these tumors vary considerably in shape and size. They are, as a rule, irregularly globular and range from the size of a hazelnut to a mass 100 centimeters in circumference. They may be single or multiple. As a rule, and especially those which attain a large size, present a lobulated surface and are attached to the parent tissue in a sessile manner. They are surrounded by a capsule of fibrous connective tissue which sends trabeculæ into the tumor dividing it into many lobules of variable size. These trabeculæ carry nutrient blood and lymph-vessels. In color these tumors vary from white to bluish gray.

On section, the cut surface presents a bluish ground-glass appearance in which white bands of connective tissue divide the tumor into various sized lobes. In consistence, the enchondroma is elastic, but this may vary from a soft and gummy character to bony hardness.

Degeneration of these tumors, due to the limited supply of nourishment of those portions of the lobules most removed from the trabeculæ which carry the blood and lymphatic supply, is quite frequent. The degeneration may be myxomatous, calcareous or fatty, and in frequency occurs in the order named. Occasionally a well-defined mass may undergo myxomatous degeneration leading to the development of a true cyst, or the whole tumor may be composed of a myxomatous matrix in which very few cartilage-cells are seen, a myxochondroma. Some specimens show the deposition of calcium salts in the matrix with a shrinking of the cartilage-cells giving rise to a type of calcareous degeneration but without bone formation. Fatty degeneration is described by some authors as an overabundance of fat droplets in the cellular cytoplasm, where it is normally present. Degeneration is more likely to occur in those tumors of the homoplastic group or ecchondromata, where the trabeculæ are not so abundant, and where there is less tendency to form lobules.

The pelvis in view of its embryologica development and numerous centers of ossification, its articulation and relation to other parts, is a not uncommon site of chondromata

and has many locations from which they may arise. These are (1) from cartilage at the symphysis pubis, (2) from the cartilage of the ischiosacral synchondrosis, (3) from the cartilaginous disc at the sacrovertebral articulation, and (4) from the cartilage of the acetabulum. The tumors arising from the above-named places are of the ecchondroma group. However, they may also arise from "cell-rests" which may be located at (1) the junction of the ascending ramus of the ischium and the descending ramus of the pubis, (2) the junction of the acetabulum and the ilium, ischium or os pubis, (3) any portion of the pelvic bones where connective tissue exists, or (4) the tumor may arise by extension of growth from the surrounding parts. These are of the nature of enchondromata.

Clark reported one case of chondroma of the pelvis occurring in a middle aged woman. This was first observed as a tumor above the hip, and in the presence of other signs a diagnosis of medullary cancer of bone was made. The patient became rapidly emaciated, anæmic, and died. At autopsy all of the os innominatum was involved in a tumor mass. The tumor measured $8\frac{1}{2}$ x 9 inches, filling the pelvis and displacing the rectum, vagina, and bladder. Microscopic examination showed the tumor to be entirely composed of cartilage and its malignancy was due to its position.

An enchondroma of the pelvis developing at the site of injury was described by Potter. The patient was a female 37 years old, who

gave a history of a fall ten months previously and two days before confinement. In falling she struck her buttocks on a stone floor. The injured area was quite painful and tender for a few months. Five months after the injury she noticed a small hard swelling on the right buttock. The mass enlarged and grew along the ascending ramus of the ischium and descending ramus of the pubis, reaching the size of a six months' foetal head. It filled the pelvic outlet causing difficult urination and defæcation and preventing intercourse. At operation the tumor had an irregular surface and was covered by a fibrous capsule. Microscopic examination showed it to be composed of a fibrillated cartilage, frequently showing several cartilage-cells in single lacunæ. Many areas of calcareous degeneration were present in the tumor.

Several other similar cases with a history of trauma at the site of tumor formation have been described by Halthouse and others. In his researches Wartmann described eight enchondromata, two from the pelvis, four from the hand, one from the lung, and one from the parotid gland. He has studied some five thousand sections of these tumors with the following conclusions. He confirms the opinion of Virchow, who indicated that enchondromata arise from connective tissue. He also claims that by a process of metaplasia the endothelium of lymph- and blood-vessels may give rise to enchondromata, and further that many chondromata arise from cartilaginous emboli within the blood-vessels.

The following are three cases of enchondro-

mata of the pelvis which have come under our observation. The first two originated in the pelvic bones while the last had its origin in the femur, and by metastasis extended to the pelvis.

CASE 1. Mr. E. H. K., age 38 years. Occupation, farmer (white). He has always had good health. Two years previously the patient received a sharp injury by being thrown on an iron rod. It did not trouble him much at the time, but later he noticed a small growth at site of injury.

On examination there was found on the inside of the left thigh and below the perineum a hard immovable growth about the size of a man's fist adherent to bone. The soft tissues over it were not involved. The glands were not enlarged. He was not inconvenienced in walking. There was slight pain which radiated toward the knee. There was no cachexia nor loss of weight. His general condition was good.

An operation was undertaken under general anæsthesia. An incision was made along the inner side of the thigh, transverse to the tumor. The tumor which was adherent to the ischium and arose from the ascending ramus was chiseled away from the bone and some of the tissue in the neighborhood was removed. Recovery was good. The patient left the hospital 41 days after entrance and thirty-nine days after the operation.

Pathological examination. This tumor was a large mass about the size and shape of a man's fist. It measured 10 x 7.5 x 6.5 centimeters. The surface was very irregular and nodular. It was composed of nodules varying in size from a pin head to a marble. The tumor was covered by a capsule of dense connective tissue of a glistening character. Scattered diffusely over the tumor mass were small lobules of yellow fat. These were bound to the fibrous tissue capsule. Over about one-fourth of the surface the capsule was missing. Here the tumor had been separated from its attachment. This part was very rough and had many fibrous

tags hanging to the surface. In many places it had the appearance of the pulp of an apple and was finely granular. This cut portion was studded with fine chalk-like areas varying in size from a pin head to a large pea or bean. These areas were very irregular in outline and of a calcareous nature.

On cutting through the tumor it was found to be composed of many distinct nodular masses of cartilage. Each mass was definitely outlined but was united to the neighboring lobules by dense fibrous tissue. The cut surface was of bluish gray color and had a finely granular or frosted appearance. In the center of several of these there were irregular chalky masses of calcification. Throughout the tumor there were thick strands of dense white fibrous tissue.

Microscopic sections of the tumor showed it to be mainly composed of cartilage and fibrous tissue. The latter save for the main trabeculæ was small in amount and passed through the mass in all directions, dividing it into small lobules. This connective-tissue stroma was loosely arranged and contained a relatively small number of nuclei.

In some parts of the sections the lobules of cartilage were mainly composed of a clear hyaline matrix in which the cells were sparse. The cytoplasm of the cells was contracted leaving almost vacant lacunæ save for the centrally placed nucleus. Again in other places the cells were more abundant and the matrix less in amount. This did not have the appearance of mature hyaline cartilage, but had a streaked appearance due to prominent fibers in the matrix. Here the cartilage was not arranged in the well-defined lobules, but had an irregular architecture with ill-defined borders. At times it was difficult to differentiate true cartilage-cells from the fibrous connective-tissue cells of the trabeculæ.

At the border of the sections and adjoining the fibrous stroma, the cartilage-cells were lying in meshes of collagen fibers of the connective tissue, but as one advanced from the fibrous trabeculæ, the cartilage-cells took on a more mature appearance and the collagen fibers became fewer in number, gradually disappearing from the hyaline

matrix. The cell walls were very indistinct and could not always be made out. This was particularly true where the cartilage was more mature. In other parts of the specimen, as many as ten cartilage-cells were surrounded by a single indented capsule. Diagnosis: Chondroma of the ischium.

CASE 2. Mr. Jos. B., age 52 years. Occupation, carpenter (negro). Patient had been in good health until 18 years ago (34 years) when he had typhoid fever. He made a good recovery and was perfectly well until five years ago (47 years) when he had trouble with his bowels and was compelled to use purgatives. Gradually, but slowly, constipation became more marked. Two years ago he had frequency of micturition, associated with some pelvic pain. Gradually, but slowly, his condition became worse. Six months ago he noticed a hard firm enlargement above spine of the left pubic bone. Being a carpenter he attributed the development of the mass to injury occasioned by the use of an auger. This tumor gradually became larger. One month ago he noticed a similar mass to the right of the pubis. He progressively became more constipated and urination was more frequent. He has not lost much (if any) in weight.

On examination he appeared to be a fairly well-nourished man. A firm tumor was found projecting above the pubis on either side and filling up the anterior portion of the pelvic cavity. The finger could be inserted into the rectum for a distance of about one inch. There appeared to be but a small space posteriorly for the bowel. Examination elicited no tenderness.

A large nodular mass, composed of numerous lobules of bluish white color was removed from the posterior surface of the horizontal rami of the pubic bones. The tumor extended laterally on both sides of the symphysis. The patient made an uneventful recovery.

Pathological examination. The tumor mass was very irregular and nodular and composed of various kinds of tissues. It had the shape of a large potato and measured 13 x 7 x 5.5 centimeters. The tumor was covered in an irregular manner by a fibrous

capsule to which many fibrous tags were attached. There were many thick fibrous bands running over it in various directions. In places the tumor was of firm, bony consistency, in others it was more elastic and at times it even appeared cystic. There were about thirty smaller pieces of tumor which during removal had been separated from the main mass. They varied in size from a walnut to one-third the large tumor and were similar to it in structure.

For the most part the tumor was occupied by lobular masses of cartilage, which had a dense, bluish white, glistening appearance, and surrounded by a definite fibrous capsule. On section, some of these masses of cartilage seemed to coalesce with the neighboring masses. In other parts, they existed as separate lobules and were attached only through intervening thick bands of fibrous connective tissue. These bands traversed the tumor in all directions and appeared to arise from the surrounding fibrous capsule. On closer inspection this cartilage was a bluish-gray color, it was smooth and had the appearance of frosted glass. This cartilage was quite elastic. In another portion of the tumor, there was some firm white calcified tissue, which was irregular and nodular, representing or suggesting calcification of definite cartilaginous nodules. These calcified areas could easily be broken into small nodular masses, each of which was separated from the next nodule by a capsule.

Attached to and embedded in the dense fibrous capsule was a mass of fat which was of a dull yellow color. On section through the cystic portion of the tumor, the interior was found cavitated in a very irregular manner. These cavities extended through the tumor mass in many directions. The cartilaginous substance in this region and surrounding the cavity was very soft and easily broken. In the thick bands of connective tissue were many congested blood-vessels. The tumor had in places a pink hue.

Microscopic sections of the tumor showed it to be composed of a hyaline cartilage. The cells were of various sizes and shape and lay in lacunæ surrounded by a matrix of varying consistency. Groups

of cells closely or loosely arranged were found. The cells were mostly oval, but a gradual transition, from stellate, to oval forms were also frequently seen. Some of the cells were surrounded by a capsule or thickened portion of the matrix. Others showed no capsule whatever, and still others showed a single capsule surrounding from three to twelve cells. In some of the cells a fine network was seen extending from the contracted central mass to the cell-wall. Running through the tumor were strands of fibrous connective tissue which contained blood-vessels. Several of these were seen to change by gradual transition into cartilage cells of the hyaline variety. The cartilage gradually assumed a more adult type as one advanced from these fibrous trabeculae inward. The matrix of the cartilage had a homogeneous pink hyaline appearance. In some places, however, minute fibers could be distinguished in this substance.

CASE 3. Mr. S., age 18 years. Occupation, student (white). For the clinical report I am indebted to Dr. B. Z. Cashman of the St. Francis Hospital. About five months previously a small growth was noted on the external surface of the right femur. Two months later this had extended to the inner side of the shaft. The patient lost five pounds in weight during the last few months. There had been some pain in the femur for the last month and some discomfort on the side of the right hip.

A firm mass was found over the inner part of the right thigh from trochanter to middle third. There was no pain on pressure. No change was to be observed in the overlying skin or veins. No other mass was present in the pelvis. The X-ray showed an egg-shaped softening below the greater trochanter.

On January 21, 1915 a small piece was excised for examination, and a diagnosis of chondromyxoma was made. A notanda was added suggesting liability to recurrence.

An operation was undertaken for the removal of the growth. The mass was easily stripped from the femur and pelvis by the hand. While removing the tumor a sensation like running the hand through a bowl of cracked ice was obtained. The operation

was followed by treatment with the actual cautery. Patient discharged February 28.

March 12, 1915. One and one-half months later a mass the size of the original was found beneath gluteus maximus and attached to the femur and ischium. This mass was also removed and cauterized. April 3, 1915. No evidence of recurrence. April 24, 1915. A mass was noticed below the descending ramus of pubis and attached to the femur. Patient was referred to an X-ray expert, who treated the case for four weeks with X-rays. The mass became softer but larger. The patient then sustained a pathological fracture of the femur. A sinus developed and blood drained from the area of injury. July, 1915. The patient was treated at Baltimore for 43 hours continuous radium. The mass continued to grow. October 20, 1915. Patient home. Marked œdema and redness over the mass which appears smaller. General health somewhat improved. February, 1916. Patient died. No autopsy.

Pathological report (January 21, 1915). There were received numerous small irregular pieces of soft cartilaginous tissue, representing a tumor about the head of the femur. The largest of these masses was 4 x 3 x 2.5 centimeters. These pieces of tissue were of a pale translucent appearance. The tissue was of the peculiar consistence of young cartilage and was quite friable. Running through it were strands of fibrous tissue forming trabeculæ and carrying small blood-vessels. The tissue was of a uniform structure and appearance. The cut surface was smooth, glassy and firm. No areas of necrosis were observed. The tissue was distinctly translucent and in the smaller pieces which were very numerous and sometimes blood stained, the fibrous trabeculæ stood out quite prominently as opaque, white strands. A firm stroma was seen to penetrate the solid gelatinous substance from these trabeculæ. In many of the smaller piece the surface was gritty, owing to the presence of small particles of calcium salts. One small piece not over 1 centimeter in diameter consisted chiefly of compact cancellous bone. This was covered on one surface by the cartilaginous tissue.

Microscopic. Sections of the tumor mass showed it to be composed of a matrix of a hyaline character, in which many cartilage-cells were found. These cells were of irregular size and shape, some being oval, large and multinucleated. Many were spherical having a wide protoplasmic ring and contracted lacuna. Others were rather spindle shaped or stellate. No mitotic figures were observed. The cells were rather loosely distributed and nowhere were they arranged in compact groups. The intervening substance was hyaline and appeared to be soft and gelatinous. It had not the solid consistence of the normal matrix of cartilage. Diagnosis: Chondromyxoma of femur. Notanda: The very soft character of the tumor and the variety of cartilage-cells, within it indicate the immature nature of the tissue. Tumors of this nature arising from cartilage have been shown to recur after removal.

Pathological report (February 2, 1915). There was received a 500 cubic centimeter jar full of cartilaginous masses or nodules looking like broken meat jelly. These pieces were similar to those described before. The largest measured 9 x 7 x 2.5 centimeters and was very irregular. On section the cut surface was smooth, translucent, and cartilaginous. The tissue was lobulated and divided by numerous fibrous trabeculæ, and although it was thus well supported, it was exceedingly friable. When the surface of the tumor was slightly scraped to remove the moisture, it appeared much like smooth, finely frosted glass. It was, therefore, dull and translucent.

Microscopic. Sections of the tissue showed a structure resembling cartilage, save that the matrix appeared less dense and contained an irregular reticulum. The cells were irregularly scattered, some small, others large, like cartilage-cells. In places again these cells were not contained within lacunæ, but appeared spindle shaped like myxoma cells. Here and there were found trabeculæ, which appeared to shade off, in gradual transition, to the tumor tissue. The trabeculæ appeared more cellular than the cartilaginous tissue. The blood-vessels

were quite thin walled, although definite arteries were encountered. Mitotic figures were not seen. Diagnosis: Chondromyxoma of femur.

In our own first two cases we see that the tumors have occurred in healthy males, laborers by occupation. Their ages were 37 and 47 respectively when the tumors were first noticed. The tumors developed after and at the site of a definite injury, in one case after a sharp trauma and in the other after a more prolonged and milder injury. Both arose from the same portion of the pelvis. They were both removed by a surgeon and showed no evidence of recurrence. Microscopically they were seen to be true enchondromata, one showing calcareous degeneration. The cells were similar to those of adult hyaline cartilage and the tissue farther removed from the trabeculæ had the typical appearance of hyaline cartilage. In those places farthest removed from the trabeculæ where the supply of nourishment was limited, the intervening hyaline substance was at times greater in amount and of a myxomatous nature. In each of these tumors the various steps of transformation (metaplasia) of connective tissue into cartilage was seen.

The third case occurred in a young man 18 years of age, giving no history of trauma, and arising primarily on the innerside of the femur. A section of this growth showed the cartilage-cells to be embryonic in type and the intervening substance was soft and gelatinous. The diagnosis of the condition in the tissues, on the first occasion of removal, was accompanied by a note stating that "tumors of this

TABLE I.—CASES COLLECTED BY AUTHOR

Author	Year	Sex	Age	Trauma	Locations
Letenneur	1855	M.	32	Definite history	Right ilium, trochanter and femur.
Parise	1862	M.	37	No definite history	Rami of pubis and ischium (left).
Molinier	1865	F.	34	No definite history	Sup. part of os ilium and sacroiliac synchondrosis.
Francois	1865	F.	35	No definite history	Region of the buttocks
Weinlechner	1875	M.	62	Not stated	Sacrum and great trochanter of femur (right).
Bayer	1903	M.	44	Not stated	Left (body of pubis) symphysis.
Bayer	1903	M.	33	Not stated	Right ilium.
Opokin	1906	F.	33	Not stated	Left pubis.
Korte	1911	M.	43	Not stated	Symphysis pubis and ilium (right).
Nathan	1914	F.	23	Not stated	Rami of pubis and ischium.
Author	1916	M.	37	Definite history	Ramus of ischium (left).
Author	1916	M.	47	Definite history	Body of pubis at symphysis.

nature arising from cartilage have been shown to recur after removal." Six weeks later the tumor involved the pelvis. We have included this case in our report to illustrate the quality of malignancy which may at times be associated with this type of neoplasm.

Schoppig in 1907 was able to collect 93 cases from the literature. Of these, 54 occurred in women which he analyzed as follows: Twenty-two gave no obstruction to labor, while the remaining 32 gave difficulty in pregnancy. A large number encroached so

decidedly upon the pelvis as to demand cæsarean section. Only a few cases in his series had been studied to show the influence of trauma in the development of the tumorous masses. Out of a group of eight in which inquiry of previous injury was made, there were five in which a definite relation was shown.

To the above series of 93 cases, I have been able to find 10 more in the literature and have also added 2 of my own, not including the one arising in the femur and metastasing to the pelvis (Table I).

Many different views have been held by the various authors as to the origin and development of enchondromata and of these we will discuss the more important. Traumatism whether severe or mild has been claimed to be a very important factor in the causation of these tumors. In our own two cases, having their primary origin in the pelvic bones both have followed a definite injury. Letenneur makes the statement, "that enchondromata almost always develop after an injury to the bone." He also cited Virchow as holding the same opinion. Just in which manner trauma and irritation act as stimuli in the production of these tumors is not definitely known. However, in a number of reported cases there is a history of previous trauma or irritation at the site of the tumor, and in those cases where no definite history of trauma is offered, many tumors are found to have originated in areas very liable to injury. Of the 12 cases which we have tabulated, a history of injury was sought in 6 and

was found associated in 3. Schoppig, in 8 cases in which reference was made to trauma as one of the etiological factors, found 5 giving a definite history of trauma. In the other cases where no history of injury had been mentioned, the author laid particular stress upon the possibility of its presence.

Sudler reported the development of chondromata in various parts of the body, as the result of trauma. That these tumors may arise from fibrous connective tissue by a process of metaplasia seems in the light of previous studies and our own, very probable. Helmholtz accidentally induced the formation of cartilage from connective-tissue cells, by injecting a solution of sudan III into the tissues of a rabbit's ear, to study its effect on the epithelium. Two months after the primary injection, pieces of the rabbit's ear were removed for examination. Under the microscope, these showed the various stages of the transformation of connective-tissue cells into cartilage-cells and were described as follows: The connective-tissue cells first retract their processes and lose their distinct outline. The nuclei become larger and more vesicular. The interstitial substance loses its fibrillar character and stains a pale pink. The cell-body then retracts from the surrounding substance and lies free in the lacuna. Before the cell recedes and imbeds itself, it is impossible to say whether the cell is a cartilage cell or a fibroblast. One of the views advanced by the same author as to the probable explanation of the above, is that the connective tissue, by the stimulation of sudan III, has returned to the

embryonal type of mesoblastic cell with the potential qualities of forming any of the stroma elements of the mesoblastic type. Similar results were obtained by Harvey, by painting the outer surface of the aorta of rabbits with silver nitrate.

These steps in the transformation of the fibrous connective-tissue cells of the trabeculae into cartilage-cells have been observed by me in two of the cartilaginous tumors studied. Incidentally both of these tumors arose from the pelvis. Wartmann, Gibbs, and other authors have observed and described this same process in enchondromata occurring in different regions in man and other mammals. In his discussion on the origin of these tumors Wartmann states his belief that enchondromata commonly arise from connective tissue by the process of metaplasia. Many authors agree with Virchow, who held that enchondromata may develop from embryonic "cell-rests." These islands of cells from the primary mesoderm develop to a certain stage of specialization and then remain in a latent state during the development of bone, and later in life under the proper stimulus give rise to one of the many cartilaginous tumors. He further states that these "cell-rests" and enchondromata are more likely to make their appearance following rickets, where ossification and growth of bony and cartilaginous parts are much retarded.

Enchondromata may also result from an inclusion of cartilage-cells (anlage), during the development of certain organs. Those likely to occur in the pelvis are enchondroma-

ta of an undescended testicle or an ovary. These organs are developed in close apposition to the vertebral column and may include an anlage of cartilage from the intervertebral discs, which later may take on the power of growth and give rise to a tumor. This was shown by Virchow to be the probable source of enchondromata of the parotid gland in which an "anlage" or inclusion was obtained from the branchial arches.

Francois reported a case of enchondroma of the ovaries occurring in a patient 74 years of age, but does not give any view as to their mode of origin. On the other hand, Wartmann in his classical researches upon enchondromata has observed and described the endothelium of the blood- and lymph-vessels retroverting to a type of embryonic mesodermal tissue from which it then developed into cartilage-cells and formed tumors.

Enchondromata of the pelvis usually develop during the third and fourth decade. Of the 60 cases reported in which attention was called to the age of the patient, 46 have occurred between the age of 20 and 40 years, and 29 of these between 30 and 40 years. Considering these tumors in the order of their frequency in particular locations, those arising in the pelvis stand fourth, the bones of the hand, femur, and tibia respectively being more frequently involved in the order named. In these latter locations, they very often are the late outcome of arthritis and particularly of arthritis deformans. Heredity seems to play little or no part whatever in the development of chondromata, although those

occurring on long bones have been found in three successive generations. These tumors occur about equally in both sexes. Of 105 cases reported, 59 occurred in females.

Considering these tumors in regard to their rate of growth and duration one can say very little, as they are usually removed surgically at an early period. However, one can say that they are, as a rule, of slow growth, but may suddenly take on the qualities of rapid growth and cause the death of the patient. Livert in his statistics on enchondromata in general, taken before the days of modern surgery, reports 12 cases lasting from one month to two years before death, 11 cases from two to ten years, 12 cases of ten to twenty years' duration, and 3 cases more than forty years' duration.

Many enchondromata of the pelvis are clinically malignant, mainly because of their position in relation to the various pelvic organs. This was the more true before the days of antiseptic and aseptic surgery, when the tumors were allowed to develop to an enormous size. Some of these tumors possess characters of true malignancy as is shown in one of our tumors having metastases. This process, it is pointed out by Wartmann, takes place by the development of buds, which grow into the lumina of vessels, are broken off and carried to distant parts. The organ most frequently involved is the lung. Some of the tumors spread to distant parts by continuity and in this way show their malignancy. This is more apt to occur in tumors of a soft character whose cells are

irregular in size and shape and simulate in appearance embryonic cartilage-cells. The matrix of these tumors is soft and of a gelatinous nature, often myxomatous. This is well shown in our Case 3, with metastases involving the pelvis. Francois cites a case reported by M. Dolbeau showing a metastasis of enchondroma of the tibia to the pelvis. The more cellular these tumors are, the more prone they are to develop typical sarcomatous masses of fibrous type.

Gibbs reported a case of enchondroma in the breast of a bitch, growing side by side with a sarcoma, but having no connection with it. In some instances it was impossible to differentiate the two types of cells save by special staining methods. It is possible that many cases of this kind with metastases and other signs of malignancy have occurred in the human, and hence reported as malignant enchondromata.

Enchondromata may be differentiated in the gross from similar growths by observance of the following: These tumors are definitely outlined and globular in shape, while malignant tumors are irregular in shape and outline. Pain is not so marked, if present at all, and not of the lancinating type as sometimes found in malignancy. Pain in exostoses is present from the beginning while in enchondromata it is absent until the growth is quite large. There is no involvement of lymphatics and only in rare cases are the blood-vessels engaged. Keen is of the opinion that enchondromata arising in children are usually benign while those arising in

later years are more liable to become malignant.

From our experience and study as well as that of others, it is evident that enchondromata of the pelvis most frequently arise in fibrous connective tissue and develop through a process of metaplasia following a stimulus, usually a definite injury. Furthermore, their clinical malignancy is mainly dependent upon their position, and only rarely do they adopt a sarcomatous character and show true tissue malignancy.

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HYALINE DEGENERATION OF ARTERIES

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HYALINE DEGENERATION OF ARTERIES.*

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The term hyaline is for the most part used to designate an intercellular substance of a colloidal character. The term by no means indicates a specific material, but rather an appearance which is simulated by a variety of substances. For the most part hyaline occurs in association with connective tissue fibrils, but another type is also observed to occur in and about epithelial cells. In general, hyaline consists of a structureless material disposed in droplets, bands, or diffuse infiltrations. When occurring in the vicinity of fixed connective tissue structures, it is disposed parallel to the neighboring fibers. When, however, it appears in a loose fibrous tissue it has the appearance of an infiltration with no relation in its disposition to the architecture of the tissue.

Much of the hyaline which is observed in pathological tissues appears to bear a relation to the blood vessels of the part. In this way it simulates amyloid and mucoid degeneration with which it has a relation not only in appearance, but also in some of its microchemical reactions. Under some conditions the similarity of hyaline to amyloid is very great. Its occurrence about capillary tubes as a cylinder lying just outside the endothelium suggests a manner of deposit like that of amyloid. Chemically, too, the substances appear related in that their reactions with acid stains are very similar. In fact it has been indicated by some authors that fundamentally hyaline and amyloid are identical, or even that amyloid is a chemical product of hyaline. In the absence of chemical analyses differentiating hyaline, mucoid, and amyloid deposits, we are forced to rely upon microchemical tests which, up to the present, have been applied in the nature of stains. Every one who has studied these staining

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reactions has been struck by the inconstancy of any particular stain for each of these substances.

To these rather closely-related products of degeneration is added another named by Goodall as elastoid degeneration. This substance consists of a softening of the colloid material contained in elastic fibers which have lost their specific character and no longer react to the special stains. The Weigert and Verhoff methods which normally pick out the elastic fibers with great specificity do not react with the materials found in elastoid degeneration. All gradations in the degree of degeneration may be observed by the manner in which these stains are taken up. This type of degeneration, though in its advanced state has some of the appearances of hyaline, may be readily distinguished from the intercellular hyaline associated with connective tissue structures. It is most frequently seen in the larger arteries associated with the heavy elastic bands on the outer side of the intima. Remnants of the elastic fibers are commonly found within its substance and a greater or less affinity for the specific elastic tissue stains may always be obtained.

Hyaline may make its appearance in the arteries in any of the three coats. In appearance it is similar to the hyaline found in connective tissues, save in one form which occurs in the small arterioles just underneath the endothelium. The latter type instead of occurring as an intercellular substance lying between the fibrils of connective tissue is laid down as a band-like deposit in which no fibrils make their appearance. In the larger arteries hyaline is found in the connective tissue thickening of the intima diffused as a homogeneous substance amidst the interlacing fibrils. In these large arteries hyaline is also seen in the connective tissues of the adventitia, where its occurrence is very like that observed in the stroma of the breast. The hyaline of the adventitia is related to the degenerative changes of the connective tissue in which the altered stroma becomes swollen and homogeneous. It would appear that the change in the intercellular substance of the connective tissue directly reverts to hyaline. The appearance of hyaline in the media is most

frequently found in the small arteries and arterioles. Under all conditions the appearance of hyaline in the arteries indicates a retrogressive change unaccompanied by inflammation. At times an inflammatory process has preceded the development of this deposit, but frequently, too, such antecedent tissue reaction is found entirely wanting.

There is no specific stain which differentiates hyaline definitely from other closely-allied bodies. We are dependent mainly upon the negative reactions for specific substances like amyloid, mucoid, and elastoid materials, coupled with the affinity which is shown by hyaline for acid stains. Among the latter eosin is most widely used, but with it positive reactions may also be obtained with acid fuchsin and methyl green. It is soon found, however, that many difficulties arise in attempting to make use of microchemical reactions to differentiate hyaline from allied substances. This is particularly true of amyloid, in which various gradations in staining qualities may be obtained with the known specific stains for this substance. It has been indicated by various authors (Schmidt, Klotz) that amyloid is a changeable and unstable compound, having a protein base very similar to hyaline. It is not uncommon to find that the iodine sulphuric acid reaction is indefinite or entirely wanting, while a more constant response is obtained by the use of methyl violet. All gradations may be obtained, from simple hyaline to the fully-developed amyloid in the same specimen. Schmidt believed that amyloid was but a more advanced degenerative process imposed upon an accumulation of hyaline, in which a new compound had developed. This he believed was the result of an extracellular fermentation and precipitation in the interstitial spaces. Klotz demonstrated the accumulation of fat within the amyloid deposits. This fat appeared after the amyloid was completely formed, and appeared to be loosely bound to the amyloid. The fat was suffused through this substance, forming a physical rather than a chemical combination with the base. A similar fatty deposit also occurs in association with hyaline. This is to be particularly observed in the hyaline thickenings of the intima, where a

diffuse staining by sudan may be seen. This fat is not contained within cells or their processes, but forms a diffuse deposit in this intercellular product. The fat may readily be removed by alcohol without causing any apparent change in the hyaline deposit. Here again the fatty materials are in physical combination, and do not appear to be linked as a chemical compound. We must thus appreciate that hyaline and amyloid have much in common and that some difficulty in differentiation may arise, save in the fully-developed deposits. It is, of course, to be recognized that amyloid has its peculiar sites of predilection which often distinguish it from hyaline. So, too, hyaline in the arteries occurs in positions in which amyloid is rarely, if ever, encountered.

In this study the aorta and its large branches and the arteries of the kidney, spleen, uterus, and ovary were examined. These tissues were obtained from autopsies of individuals ranging in age from birth to seventy years. In each case the group of organs was examined to observe the earliest beginning of hyaline change, as well as to determine the tissues most frequently attacked. The cause of death was various, including many acute and chronic infections, tumors, and accidents. It was found that the arteries of some organs contained hyaline more frequently than others. The arteries of the spleen, uterus, and ovary were affected in many cases, while those of the kidney in relatively few. The arteries of infants and children did not as a rule contain hyaline, even when death resulted from acute or chronic bacterial intoxications. The youngest case in which hyaline was noted was a child of three years, which died of empyema of the thorax, and showed hyaline change in the arteries of the spleen. This was unusual, for hyaline was rarely found in cases younger than fifteen years, but after this age it was a common finding.

The perivascular disposition of hyaline is more commonly observed in the ovary than in any other organ. This is due to the frequency with which hyaline is found to develop about the capillaries of the corpus luteum. Prior to ovulation

hyaline masses are infrequently found, but after this event hyaline is an almost constant deposit in the ovary. In the corpus luteum the hyaline appears soon after the discharge of the ovum during the reparative process of this tissue. When the capillary loops have invaded the structure, a deposit of hyaline forms a distinct band encircling the entire capillary. This deposit becomes more prominent with the retrogressive changes in the lutein cells. The hyaline forms a homogeneous and structureless mass devoid of cells or fibrils, and extends from the outer surface of the capillary endothelium to the stroma or its attached lutein cells. The capillary thus becomes isolated by a cylinder of colloid material. With the subsequent increase in the hyaline deposit, the capillary becomes compressed and narrowed and eventually obliterated. Likewise the lutein cells are displaced with subsequent atrophy. As the hyaline is deposited about the capillaries it appears to permeate the surrounding tissues as a fluid, subsequently to be deposited in a more solid jelly as true hyaline. Later, much of this hyaline is slowly removed and replaced by fibrous tissue, until none of it remains in the scar marking the position of the corpus luteum.

Associated with ovulation and the development of the corpus luteum the neighboring arteries and arterioles also partake of distinct change. Sohma has pointed out that the focal arteries of the corpus luteum undergo a series of transformations during the period of ripening of the ovum, until the complete repair of the corpus luteum has occurred. It is during the later stages that degenerative processes make their appearance in these arteries. Sohma observed that when the function of the Graafian follicle was complete a restitution of the actively-involved tissues took place. During this process when the tissues again assumed passive activities the arteries of the particular area showed regressive changes with atrophy of the media. The change from a well-developed muscular coat to an atrophy of its essential elements may be closely followed in the ovarian arterioles. During these degenerative changes hyaline is prone to make

its appearance. This hyaline usually appears to the outer side of the endothelial layer, forming a band which separates the endothelium from the middle coat. In the beginning it lies on the inside of the elastic lamina, but it is not uncommon to find it overstep this boundary and advance into the inner border of the media. The major amount of this hyaline band is homogeneous, yet it is possible to demonstrate connective tissue fibers and elastic fibrils which have been engulfed by the deposit. As the hyaline mass encroaches upon the media, the muscle cells disappear until but few cells lie along the border of the adventitia. In the early stages of the hyaline deposit in the arteries the masses may form crescentic layers before the completely encircling ring makes its appearance.

This hyaline in the arteries has a greater permanency than that of the corpus luteum. Some of it may become reabsorbed and replaced by fibrous tissue, but much remains as a permanent deposit. Goodall observed a similar permanency of the hyaline deposit of the vessels of the uterus. Here, however, all the arteries are subjected to repeated changes following menstruation and pregnancy. He was thus able to observe superimposed lesions which increased the degree of degenerative change of the arterial wall. Thus in the ovary the development of hyaline within the vascular coats appears to be the result of a readjustment of the nutrition of the circulation in that part of the ovary in which ovulation has occurred. Although the deposit is a regressive tissue change it is unassociated with the presence of a toxin. The manner of the formation of the deposit is suggestive of a transudate which is fluid at the beginning and later precipitated by enzymes. During the early stages the deposit may be reabsorbed and replaced by connective tissue, but where the deposit becomes more fixed its removal is not easily accomplished and permanent masses are then found in the vessel wall. Occasionally fatty materials are observed within the hyaline deposit, but we have not seen the evidence of calcification occurring directly within the hyaline mass.

After the age of thirty-five years hyaline deposits were

found in the spleen with great frequency. These were not all associated with the blood vessels, as many occurred within the stroma of the pulp substance. The hyaline in these situations appears to be directly related to a great variety of intoxications having a systemic origin, or being locally produced in the tissues of the spleen. The development of the reticular hyaline has been commented upon when occurring with tuberculosis, syphilis, chronic pyogenic infections, and various blood diseases. This degeneration is in no way associated with changes occurring in the vascular tree, nor is it proper that this hyaline occurring as a deposit about the splenic sinuses be considered comparable to the deposits in the arteries. It would appear that the connective tissue stroma of the spleen, like that of the lymph glands, is peculiarly subject to degeneration resulting from the influence of altered blood upon its tissues.

On the other hand, the small arteries within the tissues of the spleen suffer a degenerative process with hyaline in a fair number of cases. These arterial degenerations increase in frequency with advancing age. They are not commonly present in healthy subjects below middle life, but appear as a sequel to chronic intoxications and infections, as well as in association with a general arteriosclerosis. The small arterioles within the Malpighian corpuscles commonly show this type of degeneration without evidence of similar change in the larger splenic arteries. The hyaline deposits usually occur as a crescentic mass lying beneath the endothelium. It differs in its extent from similar deposits in the ovarian arterioles, where the hyaline forms a continuous band through the wall of the vessel. In general, however, the character of the deposits is quite similar. The hyaline material appears as a new substance in the arterial coat and is not the result of change of a preëxisting tissue. It is truly a deposit which appears to be secreted into the subendothelial spaces where a transformation into a more solid homogeneous substance is brought about. The hyaline deposit does not directly involve the elastic lamina, save when the quantity of the deposit mechanically encroaches upon this band.

The effect of this hyaline material in the splenic arterioles is twofold. On the one hand the lumen is encroached upon from one side of the vessel wall even to the extent of almost complete obliteration, while outwardly the pressure upon the media induced through the presence of the crescentic deposit leads to a considerable atrophy of its tissues. Although according to the definition of arteriosclerosis by Jores this arterial lesion does not fall into the category of arteriosclerosis, the effects upon the circulation both locally and systemically are the same. Fat is occasionally found to accumulate in these intimal deposits of hyaline, but here again we have missed the presence of calcareous change.

There is some evidence that these hyaline deposits may, in part, be replaced by fibrous tissue. We have, however, never found evidence of complete removal of the hyaline with restitution of the arterial wall. The hyaline deposits when occurring to a degree distorting the vessel lumen are, we believe, permanent in their effects. Associated with this vascular hyaline, neighboring tissue changes are not uncommonly found. The one most commonly present in our series has been fibrosis, with a diminution of the lymphoid elements in the Malpighian corpuscles. We have not been able to indicate any direct association of the deposit of hyaline in the ovary or kidney with that in the spleen.

In no organ has the presence of hyaline led to as much comment as in the kidney. In its tissues some have indicated an almost specific effect of the presence of hyaline upon function. The greatest importance has been allotted to hyaline appearing about the capillary loops of the glomeruli. It does appear, however, that the issue upon the pathological effect has been somewhat confused in the lack of recognition of the two types in which it occurs in these secreting structures. On the one hand, a deposit closely simulating the jelly-like secretion in the corpus luteum is found to accumulate about the tortuous loops of the capillaries as they lie in the glomerular tuft. Here one finds this homogeneous substance just outside the endothelial layer forming nodules of deposit which more or less compress the

capillary channels. The development of these hyaline masses may occur with little other vascular disturbance and appear in the absence of true inflammation within the glomeruli. No definite etiological factor can be offered as the cause for its appearance. Hyaline about the glomerular loops does not necessarily have an associated hyaline degeneration of the arteries. In fact its occurrence in the latter is less usual, save in a process of degeneration of endarteritic nodules. The other occurrence of hyaline in the glomeruli is a process, secondary to inflammatory reactions, when the capsular synechiæ show the accumulation of hyaline between the connective tissue fibrils. In this the presence of hyaline is directly dependent upon changes arising in areas of fibrosis, wherein the fibrils of the stroma undergo degeneration with an accumulation of these materials in their interstices. Under these conditions remnants of the fibrils may be readily demonstrated in the hyaline deposit.

It is obvious that the significance of hyaline is greater when occurring as a primary deposit where its presence has an influence on the capillary vessels. Under these conditions fibrosis may subsequently appear and add its effect in shutting off the circulation through the glomeruli. To what extent this glomerular hyaline influences the function of the kidney is dependent upon the number of these structures involved. In many instances the number of glomeruli with impeded circulation appears to be insufficient to have a bearing upon the total renal function. In others again, and particularly in the so-called chronic parenchymatous nephritis or chronic glomerulo-nephritis, the interference with the circulation has a secondary effect upon the nutrition of the tubular structures. Under these conditions the damage is widespread and much of the degeneration observed in the cortex is secondary to the obstructive process in the glomeruli. From the meager evidence at hand, it would appear that the deposit of hyaline about the glomerular loops is dependent upon a low-grade intoxication, rather than the local presence of bacterial infection.

Hyaline deposit in the arteries at times appears in an

annular band in the sub-endothelial tissues of the afferent glomerular arterioles. These deposits are not common, but when found are prone to arise close to the glomerulus, towards which they are directed. They resemble the deposits observed in the spleen, with which, however, they have no direct relation. The larger interlobular vessels show no individual hyaline deposits, except as a process of degeneration of a connective tissue thickening of the intima. The latter are similar to the changes occurring in nodular endarteritis of the aorta.

Our observations upon the presence of hyaline in the arteries of the uterus agree in the main with those of Goodall. In this situation the hyaline appears more commonly as a process of degeneration in a preëxisting tissue than as an isolated deposit. As Goodall has shown, the appearance of hyaline is associated with a restitution of the arteries after they have passed through proliferative processes during pregnancy. The hyaline change develops in those tissues which attempt to restore the lumen to the smaller size after their enlargement in pregnancy. The hyaline does not lie directly beneath the endothelial layer, but occupies a position in the vicinity of the elastic lamina, which by this time appears to have receded for some distance from the inner surface. Goodall found that the effect of repeated pregnancies upon the uterine arteries led to a peculiar new architecture, in which muscle tissue appeared on the inner side of the internal elastic lamina. He believed that this was an attempt to build a new arterial coat to accommodate the circulation during the inactive period of the uterus. When these changes have taken place, the hyaline band appears at first sight to lie in the media, but on closer analysis it is found to lie in the vicinity of the deep but altered intima.

True bands of hyaline deposit also occur in the smaller arteries of the uterus, and resemble those of the ovary. This type was found both in parous and nulliparous uteri. As we have never observed its presence prior to puberty, and as it appears with increasing frequency with advancing age, it is probable that the functional activity of menstruation bears

some relation to its occurrence. We have been unable to point to any extraneous etiological factor as having a bearing upon the occurrence of band-like deposits in the arteries of the uterus.

The hyaline changes observed in the aorta and other arteries of the elastic type differ considerably from those observed in the peripheral visceral arteries. In the large vessels we do not meet with the occurrence of hyaline as a deposit, in which the character of a secretion is prominent. The hyaline appears as an interstitial product of degeneration, resulting most frequently from changes in new-formed connective tissue. The endarteritic plaques of the aorta, with their pearly, translucent appearance, show abundant hyaline amidst the network of connective tissue fibers. Depending upon the age and nutrition of these plaques, the amount of hyaline change varies. The older lesions lose much of the fibrillar structure, and are replaced by a bland, frequently finely granular deposit. At other times the stroma is welded together in lamellæ, which lie parallel with the inner surface. These strands contain much hyaline material, and later become suffused with a lipoid substance. Hence, the hyaline observed in endarteritis is one associated with changes occurring in the connective tissue.

Broadly, therefore, we find hyaline a common evidence of degeneration of the arteries, and although it may be observed in all three coats, the intima is most commonly affected. The hyaline of arteries is of two kinds. On the one hand it appears as a degenerative process of connective tissue, under which circumstances nutritional changes of the vessel wall appear to be the important factor of its occurrence. This type is seen in the intimal thickenings of the aorta and arteries of the elastic type, as well as in the unique hyperplasia of the intima in the arteries of the uterus after pregnancy. On the other hand, hyaline also appears as a homogeneous band-like deposit simulating a cellular secretion and closely allied to amyloid. These deposits may be found about capillaries (ovary, kidney) and beneath the endothelium of the smaller

arterioles of the ovary, spleen, kidney, and uterus. The similarity of these deposits with amyloid bears more than a passing notice, as is indicated in the experimental results obtained by Bailey. This author found that by treating rabbits over long periods of time with cultures of *B. coli*, hyaline deposits, some of which gave the reaction for amyloid, developed in the kidney. It is probable that the hyaline masses appearing in the glomeruli of the human kidney (distinguished from the hyaline changes in glomerular fibrosis) are of the nature of tissue secretions primarily appearing in a semi-fluid state, later forming insoluble colloid deposits. This is also the probable mode of origin of the hyaline appearing in band-like masses in the arterioles of the ovary, spleen, and uterus. Under all conditions the presence of hyaline in the arteries demonstrates retrogressive changes in the vascular tissues.

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BACTERIEMIAS IN THE AGONAL PERIOD

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BACTERIEMIAS IN THE AGONAL PERIOD*

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THE presence of a terminal infection coincident with the lowered bodily resistance usually present for a variable period just preceding death has long been recognized, but much less is known of the relative frequency and type of the bacterial invasion. We are familiar with the fact that any condition which depresses or diminishes the physiologic activity and vitality of the body diminishes its ability to defend itself against bacterial invasion and so predisposes to infection. These changes are often so subtle that they escape detection and at times the vitality of the host is so lowered that no systemic reaction is manifested and the presence of a bacteriemia remains unsuspected. With these facts in mind the idea was suggested that routine blood cultures be taken in consecutive hospital cases immediately after death and irrespective of the cause of the fatal issue. It is to be remembered that the isolation of bacteria just after death gives no clue as to the time when the invasion took place. In some cases the bacteria found were an important factor in the disease, while in others they merely represented a late or agonal infection occurring at a time when the body defenses were at a low ebb.

The significance of terminal infections, particularly those which have occurred in the course of serious metabolic disturbances where no organic destruction of surface tissues has taken place, is difficult to estimate. It would appear that the bacterial invasion was spontaneous and arose in areas which naturally harbor these organisms. To say that these local tissues had suffered a lowering of vitality until their resistance was unable to withhold the migration of bacteria into the body, is only expressing in general terms our ignorance of the actual mode whereby mucosal surfaces serve as a protective barrier to bacterial invasion. The fact remains, however, that this is true, and, furthermore, that the conditions necessary for bacterial invasion do not require the death of the protective mucosal tissues. These conditions become particularly available when the general bodily state of metabolism is at a very low ebb. It was this state which we desired to study from the standpoint of the types of bacteria which would first take advantage of the lessened tissue resistance. The influence which these terminal infections have in hastening the death of the patient, we were unable to study closely; but we have no hesitation in saying that where an individual is harboring as virulent an organism in his blood stream as the streptococcus pyogenes, he is being seriously injured by it.

Great care was taken in the collection of the materials to exclude errors which might influence the interpretation of the results. The collecting outfit which we used consisted of tubes of plain serum broth, which was the culture medium used in all cases, sterile capillary pipettes, an alcohol lamp, scalpel, sterile

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swabs, and tincture of iodine. These were kept in readiness and always available. Upon the death of a patient, the laboratory was immediately notified and the culture taken. The median basilic or cephalic vein was selected as the site for vein puncture. The skin was slightly drawn to one side and an incision made beside the vein, deep enough so that upon retraction of the skin the vein would stand out prominently between the everted edges of the incision. Tincture of iodine was then applied to the surface of the vein by means of a sterile swab. The tip of the sterile sealed capillary pipette was then broken, passed through the alcohol flame, and inserted into the vein. In the majority of cases, the capillary attraction and blood pressure within the vein was sufficient to fill the pipette with one or more c.c. of blood. The culture medium was immediately inoculated, and upon returning to the laboratory, placed in the incubator. The cultures were carefully watched for the appearance of growth and were without exception transferred to blood agar within twenty-four or thirty-six hours. Where a growth was observed, the organisms were plated, then picked for pure cultures, and subsequently transferred to their respective "sets." From the foregoing, it can be seen that the technic, while exceedingly simple, offers the least possible chance for contamination, inasmuch as the site of puncture does not come in contact with anything save the sterile swab saturated with iodine and the sterile capillary pipette. In only a few cases was any difficulty experienced in the removal of blood, and in these instances, it was due to the anemic state of the patient or to the small caliber of the blood vessels in children. The blood cultures were always taken within ten minutes following death and before the body had been in any way handled. This is a most important consideration; for even with the lapse of a very brief period after death, the manipulation of the body, such as occurs during its transference to the morgue, may cause the distribution of bacteria to sites other than those present during life. In all cases the cultural results here reported were obtained from materials collected from the body as it lay in bed.

From the following table it will be seen that out of the one hundred and nineteen cultures taken, forty-two were positive, and seventy-seven sterile. Furthermore, it will be noted that the organisms isolated belong to the types which are not uncommonly found in human infections of various tissues. While the number of positive findings may seem small, two factors should be considered: (1) the time postmortem, and (2) the type of case investigated. The time postmortem is important inasmuch as the possibility of a postmortem invasion of blood stream has been practically excluded. Postmortem invasion, however, is a very variable factor, as has been demonstrated by many investigators, and the possibility of such an occurrence is not to be overlooked. The type of case is also important, since in this series, consecutive cases were cultured irrespective of the cause of death. Then, too, the number of sterile cultures would indicate that the technic employed was a dependable one, since the ordinary contaminations were not present. The frequent sterility of postmortem blood cultures has also been the observation of other investigators. Strauch, in his study of two thousand postmortem blood cultures, found that in about one-half, or nine hun-

dred and ninety-eight times, the cultures were sterile. These were taken from the heart's blood at autopsy, and on an average of fifteen to sixteen hours after death.

TABLE I.

NO.	BACT. NO.	HOSP. NO.	SEX.	AGE.	CLINICAL DIAGNOSIS.	BACTERIOLOGICAL RESULTS AT TIME OF DEATH.
1.	939	1676	F.	37	Fibromyomata of uterus	No growth
2.	943	1820	M.	7	Ac. append. and peritonitis	No growth
3.	950	1936	F.	37	Puerperal septicemia	B. acidi lactici
4.	951	1748	M.	66	Pneumonia—Cardiac dilata- tion	No growth
5.	968	1686	F.	48	Cholecystitis—Cholelithiasis	No growth
6.	972	1995	M.	39	Perforated gastric ulcer	Strept. salivarius
7.	999	2051	M.	69	Apoplexy—Chr.int.nephritis	No growth
8.	1013	2229	F.	24	Died during labor	No growth
9.	1017	2166	F.	28	Puerperal septicemia	No growth
10.	1021	2139	M.	16	Pneumococcic meningitis	Pneumococci
11.	1036	1996	M.	39	Sarcoma of lymph glands	Unidentified Gram-neg. bacillus
12.	1045	2019	M.	58	Carcinoma of prostate	No growth
13.	1065	2307	F.	35	Tubo-ovarian abscess	Staph. pyogenes aureus —Staph. albus
14.	1066	1804	M.	25	Lymphosarcomatosis	No growth
15.	1089	2131	M.	38	Ac. card. dilatation—Cirrho- sis of liver	No growth
16.	1090	2295	M.	37	Chr.endocarditis—Coronary embolism	Strept. p y o g e n e s— S t a p h. pyogenes aureus
17.	1098	2028	M.	36	Malaria—Sec. anemia	No growth
18.	1113	2349	M.	69	Cirrhosis of liver	No growth
19.	1117	2500	M.	44	Lobar pneumonia	No growth
20.	1121	2484	M.	24	Chronic nephritis	No growth
21.	1158	2541	M.	30	Chr. int. nephritis	No growth
22.	1175	2567	M.	21	Rupture of small bowel	No growth
23.	1193	2864	M.	65	Chr. endocarditis	No growth
24.	1229	3050	M.	31	Bronchopneumonia	Staph. pyogenes aureus
25.	1247	3045	M.	25	Typhoid fever	B. typhosus
26.	1270	2921	M.	49	Miliary tuberculosis	No growth
27.	1269	3109	M.	10	Supp. otitis media and men- ingitis	No growth
28.	1281	3135	F.	42	Pyosalpinx—Peritonitis	No growth
29.	1282	2968	M.		Diffuse peritonitis	Strept. viridans — Mi- crococcus?
30.	1332	2963	M.	36	Myelogenous leukemia	Strept. pyogenes
31.	1328	3342	M.	16	Hydrophobia	No growth
32.	1333	3381	M.	40	Lobar pneumonia	No growth
33.	1348	3391	M.	33	Delirium tremens	No growth
34.	1537	3395	M.	52	Ac. miliary tbc.	Strept. mitis—B. acidi lactici
35.	1371	3320	M.	53	Chr. myocarditis.	No growth
36.	1373	3479	F.	7 mos.	Intestinal obstruction	No growth
37.	1390	3420	M.	33	Pneumonia and tuberculosis	Pneumococcus
38.	1402	3374	F.	36	Hyperthyroidism—Thyroid- ectomy	No growth
39.	1416	3551	M.	66	Lobar pneumonia	Strept. salivarius
40.	4	3753	F.	45	Hypernephroma of ureter	No growth
41.	27	3874	M.	32	Fracture pelvis—Traumatic ileus	No growth

TABLE I.

NO.	BACT. NO.	HOSP. NO.	SEX.	AGE.	CLINICAL DIAGNOSIS.	BACTERIOLOGICAL RESULTS AT TIME OF DEATH.
42.	28	3750	F.	42	Fibroid uterus — Cholelithiasis	No growth
43.	45	3146	F.	57	Pernicious anemia	Strept. anginosus
44.	47	3931	M.	54	Lobar pneumonia	Strept. salivarius— Strept. mucosus
45.	68	3940	M.	33	Lobar pneumonia	Pneumococcus
46.	69	3856	F.	47	Cholelithiasis	No growth
47.	70	3713	F.	18	Chr. arthritis—Ac. nephritis	No growth
48.	73	3956	M.	64	Lobar pneumonia	Pneumococcus — Staph. albus
49.	98	3897	M.	60	Ac. retention—Stricture of urethra	No growth
50.	102	4091	F.	84	Cerebral hemorrhage	No growth
51.	103	4092	M.	5	Hernia	No growth
52.	116	4207	M.		Lobar pneumonia	Pneumococcus
53.	127	3672	M.	47	Nephritis and pneumonia	Pneumococcus
54.	128	4213	F.	76	Strangulated hernia—Peritonitis	Strept. mitis—B. pseudo-diphtheria
55.	149	3717	M.	40	Chr. interstitial nephritis	No growth
56.	158	2311	F.	47	Pyosalpinx and pelvic abscess	No growth
57.	181	4485	M.	70	Lobar pneumonia	Pneumococcus
58.	184	4535	M.	55	Lobar pneumonia	Pneumococcus
59.	196	4405	F.	63	Complete prolapse of uterus	No growth
60.	213	4261	F.	51	Systocele—Rectocele	No growth
61.	214	4688	M.	25	Pulmonary tuberculosis	No growth
62.	216	4617	M.	34	Cerebrospinal lues	No growth
63.	231	4750	M.	35	Tumor of base of tongue	No growth
64.	257	4836	M.	43	General peritonitis	Strept. pyogenes
65.	258	4762	F.	29	Puerperal septicemia	Strept. pyogenes
66.	285	4885	M.	20	Septicemia	Strept. infrequens
67.	300	5086	M.	6	General peritonitis	No growth
68.	303	5032	M.	44	Lobar pneumonia	No growth
69.	306	5072	M.	53	Lobar pneumonia	No growth
70.	312	4293	M.	24	Broken back	No growth
71.	340	4866	M.	46	Appendiceal abscess — General peritonitis	Strept. pyogenes
72.	347	5315	M.	35	Lobar pneumonia	No growth
73.	368	5390	F.	2	Fecal impaction	Staph. pyogenes aureus
74.	369	5288	M.	41	Lobar pneumonia	Pneumococcus—Strept. pyogenes
75.	392	5253	M.	26	Pneumonia	Pneumococcus—Strept. pyogenes
76.	423	5491	M.	32	Cholecystitis—Appendicitis	Strept. pyogenes
77.	427	5493	M.	67	Hypertrophy of prostate	No growth
78.	448	5304	M.	72	Papilloma of bladder	Strept. equi
79.	454	5531	M.	67	Carcinoma of stomach	Strept. pyogenes
80.	459	4168	M.	44	Amebic dysentery	Strept. mitis
81.	470	5674	M.		Lobar pneumonia	Pneumococcus
82.	481	5702	M.	30	Lobar pneumonia — Pyopneumothorax	No growth
83.	482	5268	M.	5	Hemolytic anemia	No growth
84.	483	5550	M.	57	Septicemia following infected thumb	Staph. pyogenes aureus
85.	489	5910	F.	19	Puerperal septicemia	Strept. pyogenes
86.	498	5300	M.	48	Lymphosarcoma — Abscess of lung	Strept. hemolyticus Strept. viridans
87.	500	5946	M.	54	Fatty degeneration of heart	No growth

TABLE I.

NO.	BACT. NO.	HOSP. NO.	SEX.	AGE.	CLINICAL DIAGNOSIS.	BACTERIOLOGICAL RESULTS AT TIME OF DEATH.
88.	508	5947	M.	36	Chronic nephritis	No growth
89.	530	6094	M.	45	Carcinoma of stomach	No growth
90.	550	6289	M.	60	Pneumonia	Pneumococcus
91.	552	5766	F.	38	Heart disease	No growth
92.	562	6339	M.	19	Ruptured liver	No growth
93.	573	6363	M.	29	Acute alcoholism	No growth
94.	576	6283	M.	53	Diabetic gangrene of foot	No growth
95.	589	6257	F.	46	Dermoid cyst of ovary	No growth
96.	607	6441	M.	61	Aneurysm of aorta	No growth
97.	608	6156	M.	33	Alcoholism—convulsions	No growth
98.	666	6195	M.	40	Amebic dysentery	No growth
99.	669	6121	F.	67	Chronic pancreatitis	Staph. pyogenes aureus
100.	671	277	M.	72	Heart, kidney, and vascular disease	No growth
101.	690	6199	M.	44	Brain tumor	Pneumococcus
102.	709	511	M.	47	Pneumonia?	No growth
103.	714	2214	M.	21	Fracture 12th dorsal ver- tebra	No growth
104.	736	489	M.	26	Peritonitis—Ventral hernia	No growth
105.	737	602	F.	48	Intestinal obstruction	No growth
106.	742	205	F.	35	Tuberculosis of spine	No growth
107.	743	189	F.	58	Acute suppurative cellulitis of hand	Strept. salivarius— Staph. albus
108.	755	481	M.	36	Multiple burns	No growth
109.	762	6206	F.	39	Peritonitis following ap- pendectomy	No growth
110.	763	194	M.	49	Carcinoma ampulla of Vater	No growth
111.	765	6540	M.	37	Abscess of liver	No growth
112.	770	639	F.	5	Meningitis	No growth
113.	772	658	F.	13	Peritonitis following ap- pendectomy	No growth
114.	773	887	M.		Dislocation of hip	No growth
115.	774	905	M.	40	Pulmonary tuberculosis	Pneumococcus
116.	782	865	F.	62	Acute dilatation of heart	No growth
117.	784	956	M.		Heat stroke—Alcoholism	No growth
118.	785	925	M.	4 mos.	Plastic operation—Harelip	No growth
119.	791	792	M.	45	Pneumonia?	No growth

From the table it will be noted that of the forty-two positive cultures, thirty-one showed the presence of one organism, and eleven showed two organisms.

The frequency of the various types of organisms was as follows: streptococcus hemolyticus alone nine times, in all thirteen times; streptococcus viridans alone four times, in all ten times; pneumococcus alone eleven times, in all fourteen times; staphylococcus pyogenes aureus alone four times, in all six times. Staphylococcus albus was not found alone, but with other organisms, three times; B. acidi lactici alone once, in all twice; B. typhosus and an unidentified Gram-negative bacillus once. From the results obtained, it will be seen that the streptococcus was the most frequent organism isolated and the pneumococcus next. This was also the observation of Strauch whose results were as follows: of the one thousand and two positive cultures, streptococci were found alone four hundred and sixty times, in all five hundred and forty-eight times; pneumococci alone one hundred and fifty-five times, in all one hundred and ninety-seven times;

colon bacilli alone one hundred and thirty-two times, in all one hundred and thirty-seven times; staphylococci alone ninety-five times, in all one hundred and thirty-eight times; paratyphoid bacilli alone fourteen times, in all sixteen times; pneumo-bacilli (Friedlander) alone ten times, in all fourteen times; bacilli emphysematous (*B. welchii*) alone twice. Of the two thousand cases examined, the blood, therefore, was positive in 50.1 per cent; and in eight hundred and eighty-one times there was one type of bacterium, that is, 87.9 per cent of all positive findings; mixed infections of two types occurred one hundred and fourteen times, that is, 11.4 per cent; and a mixed infection of three types occurred only seven times, or 0.4 per cent of all cases.

As we have observed, and this would undoubtedly be substantiated in a larger series of cases, the streptococcus is the most frequent terminal invader. The occurrence of streptococcus pyogenes in cases 74 and 75, with the pneumococcus, is extremely interesting, and further careful study, in fatal cases of pneumonia, might show that the presence of such a coincident infection is relatively frequent.

In the course of the compilation of this data it occurred to us that a comparison of the bacteriological findings of the blood before and after death would be particularly interesting, in view of the fact that similar results would confirm the provisional antemortem bacteriological diagnosis, while the finding of additional organisms, would more firmly establish the more or less theoretical supposition of "agonal" or terminal infection. In reviewing the bacteriological reports extending over the period of investigation it was found that only fourteen antemortem blood cultures had been requested upon the one hundred and nineteen cases here studied. This is strikingly significant when we consider that in this brief series forty-two positive cultures were found. It further emphasizes that either the bacteriemias existed when the clinical manifestations of them were apparently not of sufficient import to justify the requisition of a blood culture, or that the infection had occurred very late in the progress of the disease when its presence had no clinical bearing. Unquestionably, bacteriemias, and often-times fatal ones, do exist where they are unsuspected. This, I believe, would be found particularly true in surgical cases where there is often a post-operative rise in temperature and an absence of the other generally recognized signs of blood stream invasion. However, it is not our purpose to discuss those bacteriemias existing for a considerable time before death.

We have long been familiar with the fact that overwork, previous infection, malnutrition, diet, intoxication, exposure, and trauma frequently lower the bodily resistance and predispose to infection. Why, then, should we not assume that morbid conditions in general, particularly immediately before death and when the bodily vitality is at its lowest ebb, offer the most favorable opportunity for a general bacterial invasion? This supposition, in order to be conclusive, must of course be proved by a greater number of cases than have here been presented, but it is earnestly hoped that the work may be continued. The securing of frequent antemortem cultures would be the most logical way in which to check the postmortem results.

The following table illustrates our results before and after death:

TABLE II.

NO.	BACT. NO.	HOSP. NO.	SEX.	AGE.	CLINICAL DIAGNOSIS.	A. M. DATE.	RESULT.	P. M. DATE.	RESULT.
1.	1008 1017	2166	F.		Puerperal septicemia	Oct. 5-15	Strep. pyogenes	Oct. 7-15	No growth
2.	1057 1090	2295	M.	37	Chr. endocarditis—Coronary embolism	Oct. 13-15	Staph. pyogenes aureus	Oct. 17-15	Strept. pyogenes Staph. pyogenes aureus
3.	1209 1270	2921	M.	49	Miliary tuberculosis	Nov. 17-15	No growth	Nov. 28-15	No growth
4.	1227 1229	3050	M.	31	Bronchopneumonia	Nov. 20-15	No growth	Nov. 21-15	Staph. pyogenes aureus
5.	900 1138	2968	M.	31	Diffuse peritonitis—Acute gangrenous appendicitis	Sept. 10-15	B. lactis aerogenes	Nov. 29-15	Strept. viridans micrococcus (?)
6.	1282 1322	3342	M.	16	Hydrophobia	Oct. 31-15 Dec. 7-15	No growth No growth	Dec. 8-15	No growth
7.	1328 275	4885	M.	20	Septic arthritis	Feb. 29-16	Strep. infrequens	Mar. 3-16	Streptococcus infrequens
8.	316 333	4866	M.	46	Appendiceal abscess	Mar. 11-16	No growth		
9.	335 438	5268	M.	5	Hemolytic anemia (Aplastic)	Mar. 15-16 Mar. 18-16 Apr. 11-16	No growth No growth No growth	Mar. 18-16 Apr. 22-16	Strept. pyogenes No growth
10.	482 377	5253	M.	36	Lobar pneumonia	Mar. 29-16	No growth	Apr. 1-16	Pneumococcus Strept. pyogenes
11.	392 456	5550	M.	57	Septicemia (infected thumb)	Apr. 16-16	Staph. pyogenes aureus	Apr. 22-16	Staph. pyogenes aureus
12.	483 488	5910	F.	19	Puerperal septicemia	Apr. 25-16 (4 hrs. A. M.)	B. acidi lactici	Apr. 25-16	Strept. pyogenes
13.	489 625 643 651 765	6540	M.	37	Abscess of liver	June 1-16 June 7-16 June 9-16	No growth No growth No growth	July 3-16	No growth
14.	739 772	658	M.	13	Peritonitis following appendectomy	July 3-16	No growth	July 12-16	No growth

TABLE III.

NO.	BACT. NO.	HOSP. NO.	BACTERIOLOGICAL RESULT IMMED. P. M.	AUTOPSY NO.	TIME OF AUTOPSY	PATHOLOGICAL DIAGNOSIS.	BACTERIOLOGICAL DIAGNOSIS.
1.	950	1936	B. acidilactici	A-34-15	2 hrs.	Rupture of uterus and peritonitis	B. acidilactici
2.	1066	1804	No growth	A-38-15	10 hrs.	Lymphosarcomatosis	No growth
3.	1090	2295	Strept. pyogenes Staph. pyogenes aureus	A-40-15	12 hrs.	Heart disease and coronary embolism	Strept. pyogenes Staph. pyogenes aureus
4.	1123	2484	No growth	A-42-15	4 hrs.	Acute Bright's disease	B. acidilactici
5.	1229	3050	Staph. pyogenes aureus	A-47-15	6 hrs.	Lobar pneumonia	Staph. pyogenes aureus
6.	1247	3045	B. typhosus	A-48-15	1 hr.	Typhoid fever with hemorrhage	B. typhosus
7.	1270	2921	No growth	A-50-15	11 hrs.	Miliary tuberculosis	No growth
8.	1332	2963	Strept. pyogenes	A-52-15	4 hrs.	Chloroma	Strept. pyogenes
9.	1328	3342	No growth	A-53-15	2 hrs.	Hydrophobia and acute enteritis	No growth
10.	1333	3381	No growth	A-55-15	2 hrs.	Hypostatic pneumonia	No growth
11.	1348	3391	No growth	A-56-15	6 hrs.	Infected clavus of toe with septicemia	No growth
12.	45	3146	Strept. anginosus	A-1-16	1 hr.	Pernicious anemia with cholecystitis	Strept. anginosus
13.	224	4691	No growth	A-10-16	2½ hrs.	Heart, kidney, and arterial disease	No growth
14.	231	4750	No growth	A-11-16	12 hrs.	Sarcoma of tonsil	No growth
15.	340	4866	Strept. pyogenes	A-14-16	3 hrs.	Appendiceal abscess with gen. peritonitis	Strept. pyogenes
16.	459	4168	Strept. mitis	A-19-16	10 hrs.	Amebic dysentery with chronic parenchymatous nephritis	Strept. mitis
17.	530	6094	No growth	A-23-16	12 hrs.	Cancer of stomach	B. lactis aerogenes
18.	671	277	No growth	A-26-16	3 hrs.	Heart, kidney, and arterial disease	Strept. fecalis
19.	742	205	No growth	A-29-16	16 hrs.	Tuberculosis of spine	B. lactis aerogenes No growth No growth

It will be noted that in cases 2, 4, 5, 8 and 12, additional or different organisms were isolated immediately after death. Case 12, presented a very interesting finding, inasmuch as four hours before death *B. acidilactici* was isolated, whereas immediately postmortem, *Streptococcus pyogenes* was found. These cultures were both taken from the same median cephalic vein, four hours apart, and yet each yielded a pure culture. In case 1, *Streptococcus pyogenes* was isolated in the antemortem culture but was not obtained postmortem. In numbers 3, 6, 7, 9, 11, 13 and 14, the same results were obtained both antemortem and postmortem.

The value of late postmortem bacteriological findings, as obtained at autopsy, and particularly in those cases autopsied some time after death, has long been a subject of discussion. It was, therefore, believed that a comparison of cultures obtained immediately postmortem with those obtained later at autopsy would be significant.

The comparison suggested will be noted in the following table. The number, while too few to be conclusive, at least points out the possibilities of future investigations along this line. The cases, nineteen in number, were autopsied one to sixteen hours after death. Of that number, sixteen showed the same results at autopsy, while three showed further bacterial invasion. The postmortem bacterial invaders were *B. acidilactici*, *B. lactis aerogenes*, and *Streptococcus fecalis*.

CONCLUSIONS.

1. Routine blood cultures taken immediately after death reveal the presence of an unsuspected bacteriemia in about one-third of all fatal cases.

2. Streptococci are the most frequent terminal bacterial invaders of the blood stream. The pneumococcus can be isolated in practically all cases of lobar pneumonia dying before the tenth day of the disease.

3. Bacteriological findings at autopsy within a few hours after death, though fairly reliable in demonstrating the presence of organisms existing at the time of death, do not exclude the possibility of postmortem invasion.

4. The taking of frequent antemortem, immediate postmortem, and autopsy cultures is to be encouraged. Contamination may be obviated by a simple technic.

5. In the absence of adequate autopsy material, the routine taking of immediate postmortem cultures will furnish valuable information as regards the essential and terminal bacteriemias.

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